QUALITY ASSURANCE PROJECT PLAN (QAPP)

FOR LAND-BASED TESTING

April. 2012

Prepared by
<<Ballast Water Detecting Lab of Shanghai Ocean University>>

Ballast Water Detecting Lab of Shanghai Ocean University

Compiled by: Wang Qiong 2012.4.1

Name: Qiong Wang
Title: Quality Manager
Signed:
Date:

Reviewed by: Wu Huixian 2012.4.1

Name: Huixian Wu
Title: Technical director
Signed:
Date:

Approved by: Xue Junzeng 2012.4.1

Name: Junzeng Xue
Title: Lab supervisor
Signed:
Date:

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1.0 PROJECT MANAGEMENT

This QAPP describes the implementation of quality assurance (QA) and quality control (QC) activities undertaken by Ballast Water Detecting Lab of Shanghai Ocean University (SHOU-BWDL) during the land-based verification testing of ballast water management systems (BWMSs). The plan covers all aspects of QA/QC, including data quality indicators, evaluation processes, performance measures and acceptance criteria; instrument certification and calibration; personnel training requirements; documents and records; data management; and QA/QC assessments and response actions. It provides a comprehensive overview of the test facility management, infrastructure, capabilities, test methodology, measurement and data collection systems, data management systems, and includes all relevant standard operating procedures (SOPs).

The QAPP is a tool to reveal any problems before start-up and during execution of the project at as early stage as possible to minimize any potential procedural, technical and scientific inadequacies and time- and economic losses. In general, this QAPP will be used for conducting verification testing of BWMSs in accordance with International Maritime Organization (IMO) Convention for the Control and Management of Ships' Ballast Water and Sediments Convention (2004).

1.1 Title and approval page

See page 1.

1.2 Table of contents

See pages 2-4.

1.3 Distribution list

The following staff will receive a copy of this document and updated. Recipients of this QAPP are listed in Table 1.

TABLE 1. Distribution List

Names	Project Titles	Organization Names	Contact Information
Junzeng Xue	Lab supervisor	SHOU-BWDL	Email: jzxue@shou.edu.cn Phone: 86-15692166680
Huixian Wu	Technical director	SHOU-BWDL	Email: hxwu@shou.edu.cn Phone: 86-15692166682
Qiong Wang	Quality manager/ Testing personnel	SHOU-BWDL	Email: qwang@shou.edu.cn Phone: 86-13122849272
Lin Yuan	Testing personnel/ Sampling personnel/Synthesizer/ /Instrument manager/TF operation manager	SHOU-BWDL	Email: lyuan@shou.edu.cn Phone: 86-13818389140
Liang Liu	Testing personnel/Sampling personnel/Document manager	SHOU-BWDL	Email: ll_laugh@126.com Phone: 86-18817565011
Jiqiang Yang	Testing personnel/Sampling personnel	SHOU-BWDL	Email: 1137100288@qq.com Phone: 86-18301977656
Xiaolin Li	Testing personnel/Sampling personnel	SHOU-BWDL	Email: 260955213@qq.com Phone: 86-15216835202
Guiqun Liu	Testing personnel/Sampling personnel	SHOU-BWDL	Email: liuguiqun1990@163.com Phone: 86-13262975593
David Leung	Project supervisor	PACT	Email: davidleung@pactchina.com Phone: 86- 13681868346
Yvonne Xia	Technical director	PACT	Email: yvonne@pactchina.com Phone: 86- 13801914890
Jianhua Gu	Quality manager	PACT	Email: gjh@pactchina.com Phone: 86- 18602124690
Kevin Wang	Safety personnel	PACT	Email: kevin@pactmarine.com Phone: 86- 13818988552
Le Zhang	Instrument manager	PACT	Email: zhangle@pactchina.com Phone: 86- 13917335896
George Qiu	Synthesizer	PACT	Email: george.qiu@pactmarine.com Phone: 86- 15906335803
Yihua Ren	Sampling personnel	PACT	Email: renyihua@pactchina.com Phone: 86- 13795469370

1.4 Project organization

The project is a joint cooperation between vendor and SHOU-BWDL as testing site owner and testing organization.

1.4.1 SHOU-BWDL – test facility (TF) owner and testing organization (TO)

Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University was founded in September 2008. There are twenty-two persons in the lab, among which four persons are engineers and scientists with high professional title. The lab consists of sample acceptance room, hydrochemistry room, microorganism testing room, microscope room, balance room and sample storage room. The lab is dedicated to the study of the harbor ecology and invasion ecology, mainly of the ecology research study of the plankton in harbor area and ship ballast water and the microorganisms in ocean environment. This organization has published over 200 papers in

both national and international academic journals. In addition, the lab has obtained twenty patents authorizations.

The lab is equipped with all kinds of instruments and apparatus, such as BOD5 analyzer, TOC analyzer, spectrophotometer, stereoscopic microscope, conductivity gauge, turbidimeter for water micro-organism test, environmental parameters detection and plankton test. The related staff is asked to be trained before he or she conducts the testing task. The six doctors and sixteen masters are all specialized in the parameter field. By now, BWDL is able to test five organism indicators and ten water quality parameters in accordance with the ballast water discharging standards regulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments: (1) viable organisms greater than or equal to 50 μm in minimum dimension; (2) viable organisms less than 50 μm and greater than or equal to 10 μm in minimum dimension; (3) toxicogenic Vibrio cholera (serotypes O1 and O139); (4) Escherichia coli; (5) Intestinal Enterococci; (6) heterotrophic bacteria; (7) total residual oxidants (TRO); (8) dissolved oxygen (DO); (9) total suspended solids (TSS); (10) turbidity (NTU); (11) dissolved organic carbon (DOC); (12) particulate organic carbon (POC); (13) pH; (14) salinity; (15) temperature. Being realistic and creative, BWDL aims to build a competent and famous lab which is specialized in the testing of ships' ballast water in China.
SHOU-BWDL is also the owner of land-based test facility for ballast water treatment tests. For purpose of this QAPP, SHOU-BWDL is defined as the testing organization (TO) and test facility owner. Organizational structure of SHOU-BWDL is shown in Figure 1.

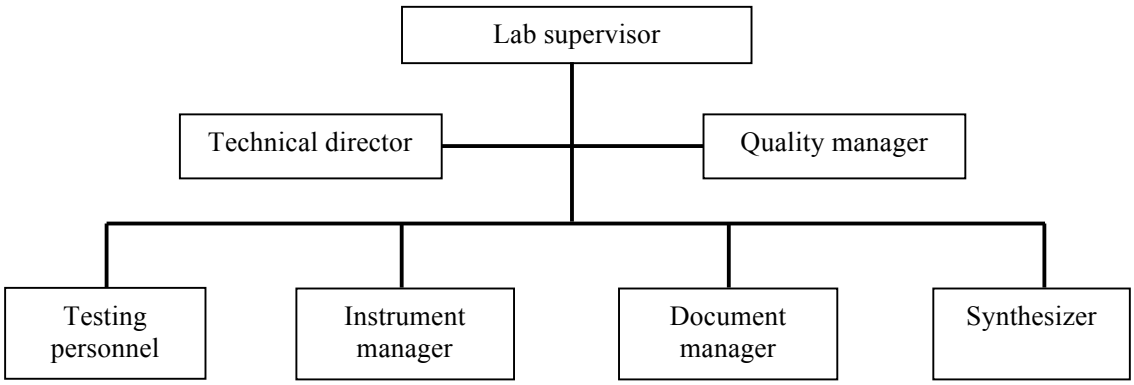


FIGURE 1. SHOU-BWDL Organizational Chart
(The above chart is a summary. A detailed explanation for the roles and responsibilities is provided below.)

Lab supervisor: Junzeng Xue

Dr. Junzeng Xue is responsible for planning and leading the overall SHOU-BWDL research agenda; keeping in touch with Zhejiang Yingpeng ship equipment manufacturing co., Ltd; approving quality system documents and standard operating procedures (SOPs); and making all final decisions on SHOU-BWDL land-based facility engineering and operational modifications and upgrades. He is also SHOU-BWDL's senior zooplankton scientist and is responsible for the supervision of SHOU-BWDL technicians in the implementation of relevant SOPs.

Technical director: Huixian Wu

Dr. Huixian Wu is SHOU-BWDL's senior microbial scientist and biological research team leader.

In the first role, she is responsible for development and implementation of the microbial-related SOPs, coordinating with SHOU-BWDL personnel to assure appropriate microbial sample collection and handling at the land-based test facility, and training relevant testing personnel for microbial samples analysis according to relevant SOPs. In the latter role, she is responsible for coordinating SHOU-BWDL biological-related research activities. She is also responsible for the overall technical works of this project and reviewing all testing results.

Quality manager: Qiong Wang

Ms. Qiong Wang is the SHOU-BWDL's Quality Manager responsible for development and maintenance of the SHOU-BWDL Quality Management Plan (QMP), SHOU-BWDL's Quality Assurance Project Plan (QAPP) and phytoplankton SOPs, and QA/QC annual report. She is also responsible for implementing all SHOU-BWDL project-specific QA/QC activities including audits and assessments, write-up of QA/QC reports on specific test activities, and assisting in the development of SOPs and project-specific QAPPs. She is also responsible for dealing with the customer complaints, and protection work related to the safety, health and environment. She also plays the role of lab internal affairs supervisor.

Testing personnel: Qiong Wang, Lin Yuan, Liang Liu, Jiayin Bian

Liang Liu is responsible for analyzing organisms ≥10 μm to <50 μm and bacteria samples, and Qiong Wang is responsible for reviewing test results of organisms≥10 μm to <50 μm. Qiong Wang is responsible for analyzing bacteria samples, and Huixian Wu is responsible for reviewing test results of bacteria samples. Lin Yuan is responsible for analyzing organisms≥50 μm, and Liang Liu is responsible for reviewing test results of organisms≥50 μm. Jiayin Bian is responsible for analyzing water quality parameters, and Lin Yuan is responsible for reviewing them.

All testing staffs conduct testing work carefully to assure the validity of the testing data. Make sure that the environmental condition of the lab meets the relevant requirements and keep the lab tidy and safety. The staff is required to participate in the training to enhance their awareness of quality importance and testing ability. The reviewers should understand the testing methods and the testing result uncertainty, and review the initial data and testing results objectively and scientifically. All the testing personnel and reviewers should keep the technical and commercial secrets of the customers.

Sampling personnel: Lin Yuan, Liang Liu, Jiqiang Yang, Jiayin Bian and so forth

The technical director is overall responsible for the field sample collection. Field sampling personnel must carry out the sampling work strictly in accordance with sampling regulations. He or she should fill in the sampling plan and sampling results, and it is field supervisor' responsibility to keep surveillance and fill in the supervision record. Responsibilities of the sampling staff include: the technical director organizes and determines the sampling plan for the organization concerned; the synthesizer is responsible for the preparation of sampling necessities and the sample acceptance, record-keeping and storage work; the sampling personnel should prepare the sample according to the sampling requirement, and collect the related data and keep records well to ensure the safety and validation of the samples. The leader of the sampling team is responsible for the management work during sampling, and he or she needs to write working summary. The technical director will organize the related staff to make a detailed sampling plan according to testing items and relevant requirement of sampling prior to collecting samples, and then verified and approved this sampling plan. The technical director convenes sampling personnel together for a meeting to arrange the tasks and explain the sampling requirements, the working contents and the work discipline. There should be no less

than two experienced staff in each sampling team, and a team leader is responsible for the field sampling management. The synthesizer is responsible for the preparation of containers, instrument, sampling list, seal, files, technical standards and letter of introduction needed for sampling. Sampling personnel needs to prepare the stuff and files mentioned above and take samples according to the sampling plan. As an integral part of the testing, the sampling personnel should keep record of the data and operation procedures for sampling. Sampling record should include the sampling procedure, identification of sampling personnel, and map of sampling locations as appropriate. There should be no less than two personnel participating in field sampling. The record kept in field should be clear, detailed, integrated, and the sampling personnel and the representative from the entrusted organization should sign on the sampling list together. The sampling personnel will seal the sample in situ, and signature of the representative from the entrusted organization may be needed as necessary. Once the samples are sealed, no one is permitted to change or make a replacement. And the sampling personnel should strictly follow the work principles to ensure the authenticity, unbiased and representativeness of the sample.

Instrument manager: Lin Yuan

As instrument manager, he is responsible for the maintenance of the instruments, keeping operation record and maintenance record of the instrument, the calibration of the instrument and preparation and custody of the instrument record card.

Synthesizer: Lin Yuan

He is responsible for preserving the testing samples in right conditions taking the requirements of the custom into consideration. He is responsible for storage and management of the consumables. He is also responsible for supervising and inspecting the storage and dispose situations of dangerous goods. And he is responsible for acceptance of the customer's testing samples and appendices and keeping record of the status characteristics of those; responsible for the test work for the external custom, sample number and status identification; store the samples required to be held in time and keep the availability and integrity of the sample in the storage period. He is responsible for the custom service, getting access to the requirements of the custom and satisfying their needs. In addition, he takes the responsibility for delivering the feedback information to the person related in order to improve the quality management system. He is in charge of dealing with the complaints and he is asked to summarize the requirements of the custom and report to the quality manager in time. He is responsible for the preparation of the facilities and environmental conditions for test. He is responsible for compiling test reports, and then the test reports are stamped and delivered by his.

TF operation manager: Lin Yuan

Mr. Yuan Lin is SHOU-BWDL's land-based test facility operation manager. In this role, he is responsible for operating the SHOU-BWDL land-based test facility, assuring that the facility is properly maintained and all equipment and supplies are in a good state. He is also responsible for the development of SOPs related to operational aspects of land-based testing, and coordinating with the project manager, Dr. Junzeng Xue, and senior researchers to assure that effectively sampling and appropriate data are collected for this project.

Document manager: Liang Liu

As document manager, he is responsible for the classification, cataloging and custody of the documents. He is responsible for the filing and managing of test reports and documents related.

One of his other responsibilities is to file and manage the technical documents such as standards, regulations, procedures and system documents and the personnel technical documents as well. Moreover, he is asked to keep the file room safe and clean and ensure that the documents in good conditions. He is also the sample keeper and responsible for the classification and record of the sample. He is responsible for keeping the environment of the sample room in normal condition and he should make the sample room safe and clean and also keep the samples in good condition. He is responsible for the distribution of the test reports in time.

The lab supervisor should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification. If anyone who has not completed training is to be assigned to finish one task, he or she will be supervised. For those who undertake specialized work, there should be qualification confirmation corresponding to their education, training, experience, specific test requirements and certifiable skills. The specific requirements are as follows: for those people who are color blindness should not undertake the tests concerning color identification. For those people who undertake the biotic experiments should know well about the knowledge of bio-test safety operation and sterilization. The chemical parameter authorized signatory should have the undergraduate degree or above in chemical, and moreover, he or she should have the technical working experience for three years at least. If not, he or she should have worked in the chemical related field for at least ten years.

1.4.2 Vendor

PACT is an equipment manufacturer and contractor in Ballast Water Treatment, established in 1998.
PACT combines decades of experience in design, manufacture and operation of water and wastewater treatment plants in a variety of industries. PACT design and process engineers are a select group of professionals that have gained their experience operating and/or managing major industrial water and wastewater treatment plants.
PACT has been certified with ISO 9001:2008 by China Classification Society (CCS) and establishes, implements, maintains and continually improves Quality Management System according to ISO 9001:2008.
PACT is headed at Shanghai with our development, sales and technical depts.. PACT's facilities locate at Wuxi and Shanghai. Good technical support and after sales service assure our good service to the clients. We provide price competitive product and good after sales service through international cooperation. Currently, we have set service offices in Hong Kong, Southeast Asia, Dubai, Greek, etc. for Ballast Water Treatment.

PACT marine™ BWMS consists of:
Filter – 40um self-cleaning filter
UV System – medium pressure UV(MPUV)
Control System – UV power panel, control panel and onlinemonitoring instrument (including flow meter, light intensityprobe and temperature transmitter)

An organizational chart for the project is shown in Figure 2.

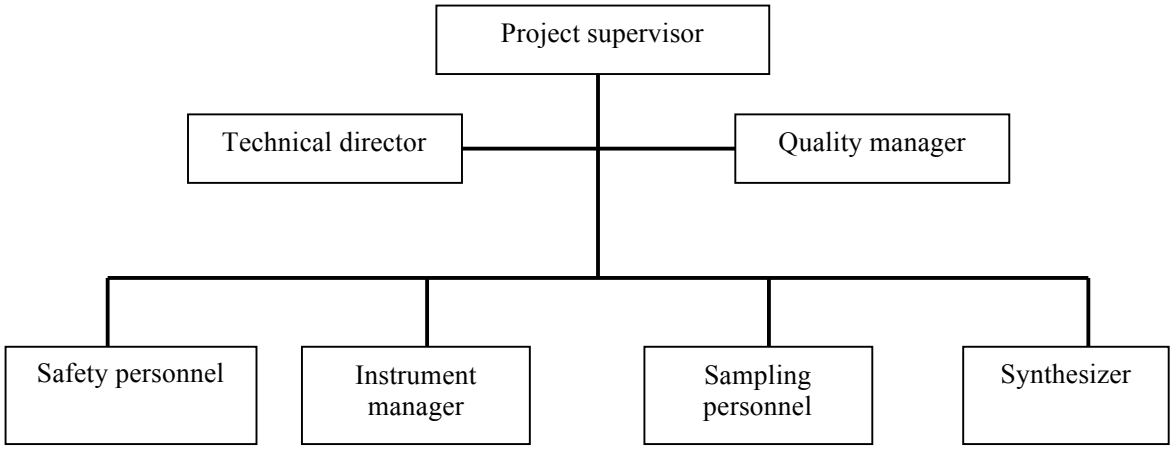


FIGURE 2. Organizational Chart of the Research and Development Organization

Project supervisor: David Leung

The project supervisor is responsible for the overall management work of the project. It is the responsibility of him to organize the human resource; material resource and financial resource of the company to ensure that the project goes on wheels. He is responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility for urging the staff of the project to follow the requirements of management system files and regulations of the company.

Technical director: Yvonne Xia

The director is responsible for the overall technical works of the project, and he is responsible for the technical training of the personnel involved in the related test work, arranging the experimental flow and technical principle studying for the related staff. Organizing and coordinating the development of the test is also one of his responsibilities. He is also in charge of dealing with the emergencies occurred during the test process. Moreover, he is responsible for the assurance of test tempo and device status to be in compliance with the requirements of the QAPP.

Quality manager: Jianhua Gu

He is responsible for the quality related work and ensures that the quality objectives to be fulfilled. In addition, he is in charge of the safety, health and environmental protection work throughout the development of the project. He is responsible for supervising the staff of the project team to finish the work in accordance with the QAPP.

Safety personnel: Kevin Wang

He is responsible for the safety of the test field. Keep eye on the safety of the test field, give suggestions on how to deal with the potential safety hazard and monitor the implementation of the improvement measures and ensure the test running in order.

Instrument manager: Le Zhang

The operation of the ballast water management system in conformation with the requirements in the test process is one of his responsibilities. And keep record of it. Report to the technical director about the running status of instruments and help the technical director with solving the defaults of the instruments.

Synthesizer: George Qiu

He is responsible for the management of the document and files over the whole course of the project. Organize and store the documents according to the requirements. He is also in charge of the coordination of the resources in field test.

Sampling personnel: Yihua Ren

He is responsible for assisting the test organizations in collecting the samples. Ensure that the samples are classified and managed in order.

1.5 Project definition/background

Ballast water discharges have historically been a major source of nonindigenous species introductions to marine ecosystems and are recognized internationally as vectors for the translocation of invasive marine organisms. The International Maritime Organization (IMO) has been actively engaged in seeking a solution to the ballast water problems. The aim of the International Convention for the Control and Management of Ship's Ballast Water and Sediments (hereafter BWM Convention), is to reduce the risk of introducing non-native species, and also to enhance protection of the marine environment and biodiversity. The BWM Convention, when ratified, will require ships to replace ballast water exchange (BWE) with ballast water treatment (BWT) using technologies such as filtration, chlorination or deoxygenation that are able to meet a numeric performance standard: ships shall discharge less than 10 viable organisms ≥50 µm per m^3; less than 10 viable organisms ≥10 to <50 µm per mL; less than 1 colony forming unit (cfu) of toxicogenic *Vibrio cholerae* (O1 and O139) per 100 mL; less than 250 cfu of *Escherichia coli* per 100 mL; and less than 100 cfu of intestinal *Enterococci* per 100 mL. One way to meet the requirements of the BWM Convention is ballast water treatment. Ballast water treatment is viewed as an important step in mitigating the proliferation of aquatic invasive species in U.S. coastal waters and the Great Lakes. The industry has responded to the expected need in treatment technology and several treatment systems are already available.

The objective of this verification testing is to evaluate the performance characteristics of commercial-ready ballast water treatment technologies with regard to biological treatment performance. Given the variety of ship and ballast tank types, and potential treatment system configurations, this QAPP addresses the use of a land-based testing facility (TF), to provide controlled conditions for verifying treatment performance.

Land-based BWMS verification testing will be conducted in a manner providing information that is comparable to the maximum practical extent, to ensure that consumers and other stakeholders can make informed choices in selecting appropriate ballast water treatment technology for shipboard installations. It is believed that BWMSs performing well under the controlled but challenging conditions specified in this QAPP at land-based TF will have a reasonable chance of performing as well in a shipboard installation.

1.6 Project/task description

1.6.1 Description of technology set-up

1.6.1.1 Components and functions of the PACT marine™ BWMS

PACT marine® P-300 is consisted mainly by:

（1）Filter --- a 40μm self cleaning filter. The filter size: 2769mm(L) *610mm(¢)
（2）UV System --- a medium pressure UV（MPUV）.
UV size: 925(L) mm *680 (W) mm* 651(H)mm
（3）Control System --- an UV switch board, a control panel and online monitoring instrument（including flow meter, light intensity probe and temperature transmitter）. HMi soft uses wincc flexible 2008。MCC size 1000(L) mm * 600(W) mm * 1900(H)mm；PLC size 600(L) * mm* 350(W) mm *1500(H)mm

Nominal Voltage/Power of the whole system assembled: 220v，50kw；380v，5kw

Considering the system assembling, especial flexibility of resembling, the filter and UV system in PACT marine® are separated. The footprint of the filter is larger and the UV system can be assembled anywhere after the filter and before deballast treatment.

The UV system, as essential, can be installed the existed pipes without any holder. The system can be assembled horizontally and vertically and its performance won't be effected accordingly.

1.6.1.2 Working Procedure

Generally, when ballast water is loaded in the ship, the seawater will flow into the sea chest, then pass a mechanical filter to remove bigger organisms and substances, and flow into the Ballast Water Treatment (BWT) last.

Ballast Process：

When ballasting, seawater will pass a coarse filter to remove bigger organisms and substance primarily, then pass a filter F-01 in Ballast Water Treatment (BWT) to remove organism larger than 40μm and solid particles. The filter can be self cleaned according to PLC without breaking off filtering. The solid particles and organisms filtered by the filter would flow into backflushed water when the filter is backflushed and then be discharged into local ocean. Therefore, active ocean organisms and sedimentation in the ballast tank are reduced and then the living and growing probability of ocean organisms in the ballast tank are reduced accordingly. The ballast water after being filtered will pass the UV System UV-01 and then flow into the ballast tank.

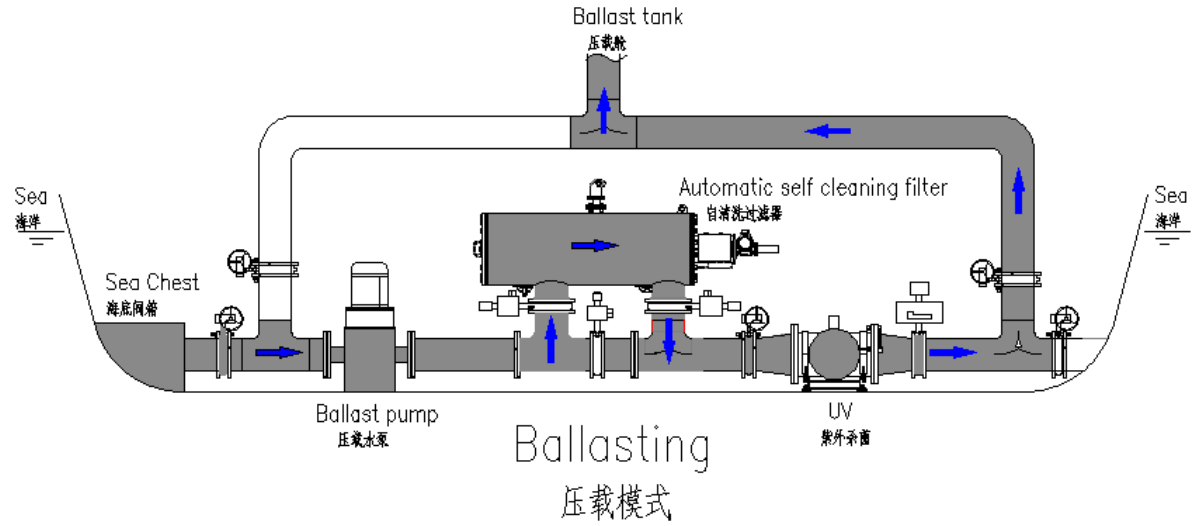


FIGURE 3. Schematic Diagram during Ballasting

Discharge Process：

When discharging, the ballast water in the ballast tank will not pass the filter but the UV System and then be discharged after treatment by the UV System. Considering the probability of ocean organisms' living and reliving by light, the ballast water should be sterilized again before discharging. PACT marine® can realize statistics auto collection and auto recording, including flow rate, light intensity and temperature.

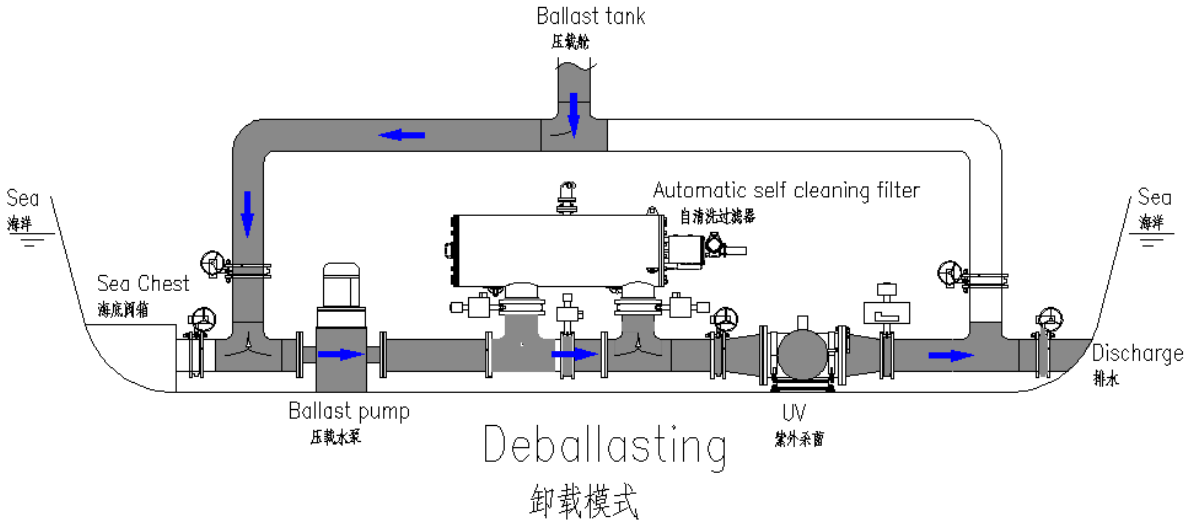


FIGURE 4. Schematic Diagram during De-ballasting

1.6.1.3 Experimental prototype

Parameters of the PACT marine™ BWMS for land-based testing (Figure 5) are as follows:

- Treatment capacity: P-300, 300M³/H
- Power supply voltage: 220V
- Filter rating: 40um
- Design pressure: 10bar
- Working pressure: 3bar

The general arrangement drawing of the system is as shown in Figure 5.

FIGURE 5. General Arrangement Drawing of the PACT marine™ BWMS

1.6.2 Description of SHOU-BWDL's land-based test facility

Land-based tests take place at SHOU-BWDL's land-based TF located in No.2 dock of Yangshan Harbor in Shanghai, China (Figure 6). Key features of the TF include:

- Ballast pump: pump the water into BWMS with a capacity of 300 m^3/h
- Sampling facility: high quality in-line sampling
- Treated tank: one 250 m^3 treated tank with concrete basin used to store the treated ballast water
- Control tank: one 250 m^3 control tank with concrete basin used to store the untreated ballast water
- Feed tank: an aeration device is installed inside of it. This tank is utilized as a container for intake water augmentation to achieve challenge water conditions required by IMO. The volume of the feed tank is 500 m^3
- Connection lines: easy plug-in connections for BWMS
- Supporting facility: connection to tap water for facility cleaning. Capacity to supply power in-situ.


金山区
嵊泗县
Hangzhou
Bay
SHOU-BWDL
land-based test facility
岱山县
G15
慈溪市
G329
G9211
定海区
舟山市
Donghai
Sea
20 km
G1501
镇海区
黄龙区

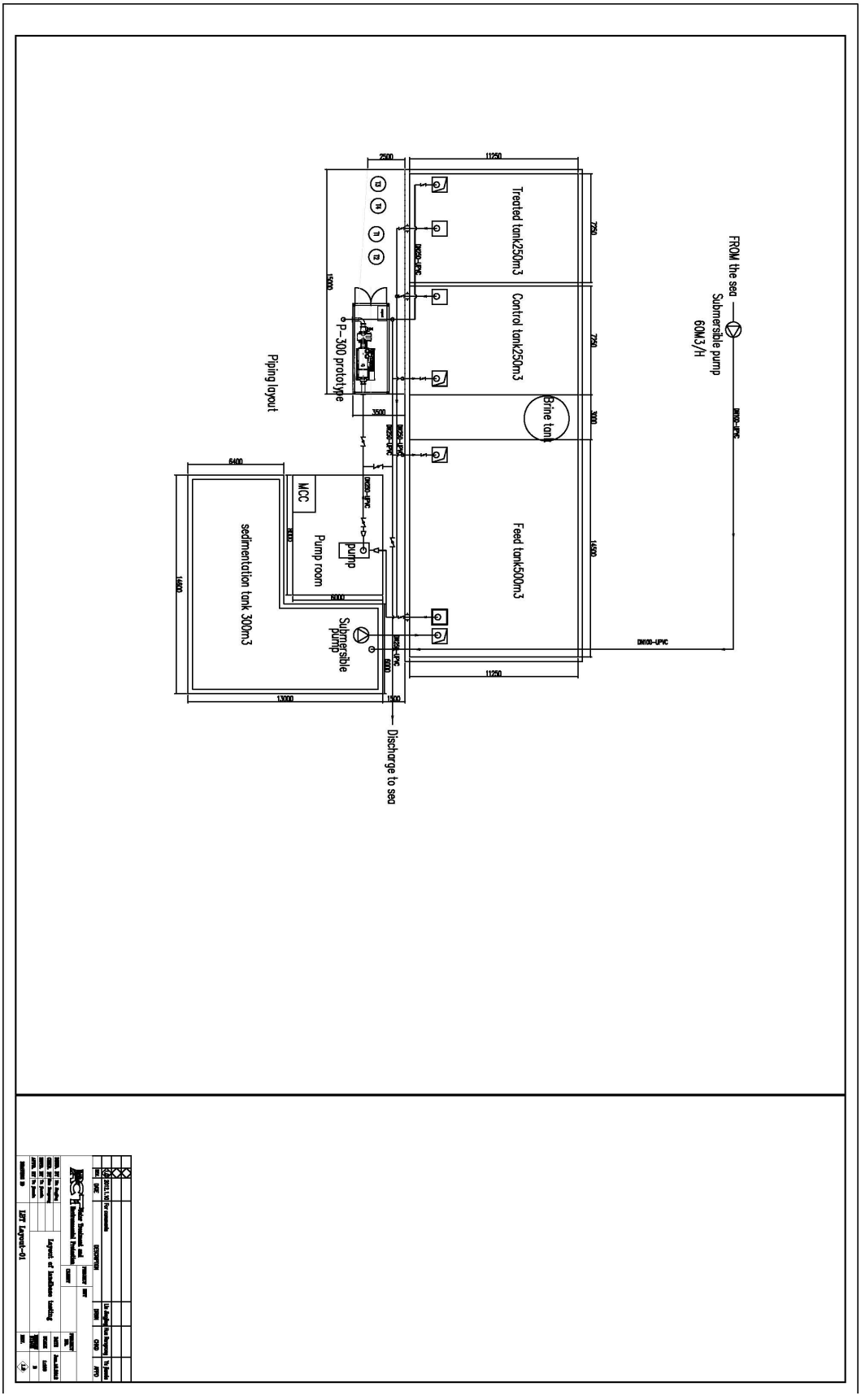


FIGURE 6. Photo of the SHOU-BWDL Land-based Test Facility

SHOU-BWDL's land-based TF draws raw intake water from Yangshan Harbor. This main flow of intake water can be augmented with organisms just prior to being split into control and treatment tracks. The influent water flows into control tank with a flow rate of 250 m^3/h and then the same influent water flows into PACT marine™ BWMS and into a 250 m^3 treated tank.

After a pre-determined retention period, water is discharged sequentially from the treated and control tanks. The water is directed either back to the harbor. The treatment track water may be passed through the treatment system again on discharge.

1.6.3 Applicable standard/criteria

1.6.3.1 Water quality of test water

With respect to physical/chemical and biological characteristics of the influent water, SHOU-BWDL's land-based intake water source naturally meets the IMO G8 requirements for intake organism densities and physical/chemical conditions (Table 2).

For those parameters that often do not naturally meet the IMO G8 requirements, SHOU-BWDL has the ability to augment intake water to achieve recommended IMO parameter levels. Frequent monitoring of water chemistry and biology is undertaken to predict valid run conditions for test trials.

Water quality parameters in Yangshan Harbor are shown in Table 2.

TABLE 2. Water Quality Parameters in Yangshan Harbor

Parameters	Units	Yangshan Harbor
Total suspended solids (TSS)	mg/L	595.3
Salinity	PSU	20.2
Organisms>50 μm	individuals/m^3	2.8×10^3 (12 species from 5 different phyla)
Organisms 10 – 50 μm	cells/mL	121 (8 species from 1 phyla)
Heterotrophic bacteria	cfu/mL	2500

Water quality requirement

According to IMO G8, for any given set of test cycles (5 replicates is considered a set) a salinity range should be chosen. At least two sets of tests cycles should be conducted, each with a different salinity range and associated dissolved and particulate content as shown in Table 3. Tests under adjacent salinity ranges in Table 3 should be separated by at least 10 PSU. Given the salinity, the test water used in the test set up described above should have dissolved and particulate content in one of the following combinations.

TABLE 3. Test Water Quality Requirements

	Salinity (PSU)	DOC (mg/L)	POC (mg/L)	TSS (mg/L)
Test water 1	> 32	> 1	> 1	> 1
Test water 2	3 - 32	> 5	> 5	> 50
Test water 3	< 3	> 5	> 5	> 50

Organisms requirement

If cultured organisms are needed, *Escherichia coli*, rotifer, artemia nauplli and microalgae will be used. The influent water utilized for biological test should meet the following criteria (Table 4).

TABLE 4. Requirement of Density of Test Organisms in Test Water

Items	Density
Organisms>50 μm	10^5 - 10^6 ind./m^3 (at least 5 species from 3 different phyla)
Organisms 10-50 μm	10^3 - 10^4 ind./mL (at least 5 species from 3 different phyla)
Heterotrophic bacteria	10^4 cfu/mL

1.6.3.2 Challenge water preparation

Water Quality Augmentation

Natural seawater from Yangshan Harbor is pumped and settled in a sedimentation tank for 16 hours. Then the water is pumped to the feed tank following addition of zooplankton, algae, and bacteria to meet IMO G8 requirements listed in Tables 3 and 4. The salinity of natural seawater from Yangshan Harbor is about 20 PSU, therefore it can be used as medium salinity water. However, high salinity water is increased using bittern.

Organism Augmentation

The marine organisms added into test water are cultured by Shanghai Ocean University (Figure 7). During the test, zooplankton (mainly artemia), algae (e.g., green algae, diatom etc.) and heterotrophic bacteria (i.e., *Escherichia coli*) in specific concentration are amended to the feed tank (Figure 8); the water in the tank is aerated by the aerating apparatus to keep the organism alive and also well-mixed. By this way, the organism concentrations in influent water meet IMO G8 requirement.

FIGURE 7. Organisms Culture

FIGURE 8. Organisms Augmentation

1.6.4 Test cycles

Test activities at the SHOU-BWDL land-based TF aims to conduct certification/verification testing, i.e., formal assessment of performance against D-2 discharge standards.

1.6.4.1 Test cycles for biological efficacy performance

The test cycles should be conducted as follows:

- The ballast water is pumped and treated in line
- The treated water and control water are stored in treated tank and control for 5 days, respectively
- The ballast water is pumped at discharge

1.6.4.2 Measures for preventing cross-contamination

Prior to each test or between test cycles, the treated tank and the control tank should be washed by the high pressure freshwater, and after that, clean them with a rag to get rid of the substances like the fragments, organisms and so on. Cleaning procedures of treated tank and control tank

should be performed as follows:

- prior to each test, fill the involved tanks with freshwater and also rinse the pipes;
- high pressure freshwater is injected to the wall of the tank from top to the bottom;
- drain the remained water in the bottom of the tank by a submersible pump;
- wipe the tank with a clean rag again until it is thoroughly clean (visual inspection);

To prevent cross-contamination between test cycles, all the sampling equipment, such as the sampling barrel, the sampling bottle, the sieve should all be washed by freshwater.

1.6.5 Test schedule

The test schedule is made immediately after the assignment of the entrustment agreement. And the test procedures will be strictly following the test schedule (Table 5)

TABLE 5. Project Schedule

Items \ Date	March 2012	April 2012	May 2012	June 2012	July 2012	August 2012
QAPP	√					
Test preparation	√	√	√			
Organisms culture		√	√	√	√	
BWMS commission		√				
Test cycles		√	√	√		
Dada determination		√	√	√		
Data analysis			√	√	√	
Testing report						√
Project summary						√

1.6.6 Test items

Test items throughout the land-based testing are listed in Table 6.

TABLE 6. Test Items

No.	Test items	No.	Test items
1	pH	9	Dissolved organic carbon (DOC)
2	Turbidity	10	Organisms 10-50 μm
3	Salinity	11	Organisms ≥50 μm
4	Temperature	12	Heterotrophic bacteria
5	Dissolved oxygen (DO)	13	*Escherichia coli*
6	Particulate organic carbon (POC)	14	Intestinal *enterococci*
7	Total residual oxidant (TRO)	15	*Vibrio cholera* (serotypes O1and O139)
8	Total suspended solids (TSS)		

1.7 Quality objectives and criteria for measurement data

The objectives of the test data are to ensure the objectiveness and scientificity of water quality parameters (environmental parameters) and organisms' parameters. Error data falling outside of the controlled range is invalid. Organism parameters before treated by PACT marine™ BWMS are: test organisms with minimum dimension greater than or equal to 50 μm which consist of at least 5 species from at least 3 different phyla/divisions in certain concentrations should be no less than 10^5 per cubic meter; overall density of the tested organisms with minimum dimension less than 50 μm and greater than or equal to 10 μm which consist of at least 3 different phyla/divisions in certain concentrations should be no less than 10^3 per milliliter; concentration of viable heterotrophic bacteria should be more than 10^4 cfu/mL. The treated water for ballasting is to be in accordance with the regulation stipulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments: less than 10 viable organisms per cubic meter greater than or equal to 50 μm in minimum dimension; less than 10 viable organisms per milliliter less than 50 μm in minimum dimension and greater than or equal to 10 μm in minimum dimension; toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 cfu per 100 milliliters; *Escherichia coli* less than 250 cfu per 100 millilitres; Intestinal *enterococci* less than 100 cfu per 100 milliliters. Viable organism concentration in discharged water from the control tank untreated by PACT marine™ BWMS meets the requirements that exceeding 10 times of the treated ballast water. The salinity (S), dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS) of the tested water should meet standards as required.

1.8 Special training needs/certifications

1.8.1 Sample collection and handling

The sampling and sample preparation personnel should be trained in accordance with the Guidelines for Ballast Water Sampling G2 and the Specification for Marine Monitoring GB17378-2007. The way to determine the needs for training and person to be trained should

meet the requirements of the Personnel Training Procedures of Ballast Water Detecting Lab of Shanghai Ocean University. The training plan should be made with reference to the project tasks currently and expected. The availability of the training is to be evaluated. During the training, the trainer should get to know every step of the sample collecting and preparing. The experienced staffs in the lab for conduct the sample collecting and preparing work should supervise the trainer.

1.8.2 Laboratory test and analysis

The test and analysis staff of the lab should be trained according to the Personnel Training Procedures of SHOU-BWDL; Specification for Marine Monitoring GB17378.4-2007, GB17378.7-2007; Water quality- Determination of free chlorine and total chlorine: Spectrophotometric Method Using N,N-diethyl-1,4-phenylenediamine HJ 586-2010; Observation Method for Gulf Ecosystem; Standard Examination Methods for Drinking Water—Indicator Microbes GB/T 5750.12-2006; Diagnostic Criteria for Cholera WS 289-2008, Water quality – Detection and Enumeration of intestinal *enterococci* - Part 2: Membrane Filtration Method (ISO 7899-2:2000); Guidelines for Approval of Ballast Water Management Systems G8; Procedure for approval of ballast water management systems that make use of active substances G9; General requirements for the competence of testing and calibration laboratories (ISO 17025-2005).

Those workers who will undertake chemical-related work in the lab should learn how to protect and rescue themselves. The important chemical test staff (those who knows well of the test methods, procedures, objectives and result assessment) should master the assessment method for determination of uncertainty of environmental parameters analysis. The organism test staff should know the safe handling and sterilization procedures of organism test. All the staff should be assured to be qualified and supervised to carry out the work according to the management system.

The lab manager should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment and test report sign.

1.9 Documentation and records

1.9.1 QAPP

The development and research organization and the test organization will discuss and determine the QAPP prior to the implementation of the project. And the QAPP will be handled and recorded as the project controlled document.

1.9.2 Field running record

To make sure that the system operates within the normal parameter range in the test cycle, running status of the ballast water management system is recorded (Table 7).

1.9.3 Field sampling record

Information about the collected sampling data will be recorded on the water proof table and the information in table is about the date, sampling personnel, weather, environmental condition, sample lot number and the identified sample quantity (Figure 9). Any deviations from the standard sample procedure or emergencies should be recorded.

Sample ID:	
Sampling time:	Temperature:
Humidity:	Sampling point:
Sampling personnel:	Supervisor:

FIGURE 9. Sample Label

The samples collected will be named according to standard identification serial numbers. The sample ID code: SHOU-BWDL- PACT -tri-digit serial number + abbreviation of the test classification + the order of one sample. The abbreviation of the test items are as follows: A represents viable organism sample with a dimension of 10~50 μm; B represents viable organism sample greater than 50 μm; C represents bacteria sample; D represents environmental parameter sample. For example: SHOU-BWDL-PACT-001A1 represents the first sample for 10~50 μm detection which collected at S1 during the first test cycle, and it was delivered by PACT Environmental Technology Co., Ltd and tested by SHOU-BWDL. Sampling record is shown in Table 8.

1.9.4 Record of chain of custody

To assure the quality control of the project, keep record of chain of custody (Table 9).

TABLE 7. Running Record of PACT marine™ BWMS

Test cycle No.		Test date			
Test location					
System		Operation mode	Ballasting □ De-ballasting □		
Ready condition record of BWMS					
Pre-startup time		Time for ready			
Fault during readiness		Fault handling situation			
Running status record of BWMS					
Start time of operating		End time of operating			
Sampling time for #1 replicate		Sampling time for #2 replicate		Sampling time for #3 replicate	
Fault situation during operation		Fault handling situation during operation			
Record of BWMS operating parameters					
Total power (kW)		UV lamp power (kW)			
Recording time					
Flow (m^3/h)					
UV irradiation (mW/cm^2)					
Percentage of UV power output					
Inlet pressure of filter (bar)					
Outlet pressure of filter (bar)					
Inlet temperature of UV lamp (^oC)					
Outlet temperature of UV lamp (^oC)					
Remarks:					

Recorder: Supervisor: Supervisor of CCS:

TABLE 8. Record of Sampling Result

No._____

Sample name			
Sampling date		Sampling location	
No. of sampling point		Sampling time for #1 replicate	
Sampling time for #2 replicate		Sampling time for #3 replicate	
Numbers of samples		Environmental factors	Temp. °C Humidity %RH
Sampling results	Organisms ≥50 μm		
	Organisms 10 ~50 μm		
	Bacteria:		
	Water quality parameters:		

Recorder: Supervisor: Supervisor of CCS:

TABLE 9. Record of Supervising

No._____

Task number		Custody date	
Test item		Supervisor	
Test objective			
Testing personnel			
Custody content			
Contract (letter of authorization)	Complete □ Lack of contents □ None □		
Test plan	Complete □ To be □ None □		
Instrument	Calibration status Qualify □ Allow to use □ Forbidden to use □		
Personnel	Qualification With qualification license □ Without qualification license □		
Samples	Quantity		
	Appearance quality Qualified □ Unqualified □		
	Unique mark Yes □ No □		
	Sample disposition record Yes □ No □		
Test method	SOP Right □ To be completed □ Faulty □		
	Standard (Regulation) Yes □ No □		
Environment	Temp. °C	Humidity %RH	
	Condition Meet the requirement □ Not meet the requirement □		
Record	Original record Meet the requirement □ Not meet the requirement □ None □		
	Service record of instrument Meet the requirement □ Not meet the requirement □ None □		
Response of custody			
Remarks			

1.9.5 Laboratory original data records

Original records are shown in Appendices A-O, respectively. The original data in the lab must be recorded clearly, and the records will be stored in an appropriate facility to keep them away from damage or losing, and also be accessed easily. Preservation period of the record must be specified and all the records should be kept secret. The collection, retrieval, access, file, storage, maintenance and cleaning of the quality record and technical record should be in compliance with the Control Procedures of Ballast Water Detecting Lab of Shanghai Ocean University.

The lab should preserve the detailed records of the information about original observation, educe of data, verification route, calibration records, personnel record and the copies of report distributed within the stipulated preservation period. Information such as sample number, test date, standards, and test conditions and so on should be included on each test record or calibration record. This would be of great help to identify the factors of uncertainties and assure the repeatability of the test or calibration in conditions similar to the original. The content of the records should involve information about the name of the sampling personnel, the test personnel, verification personnel. If culture medium is prepared, it is necessary to make a record of the name and type of the culture medium; marks of the preparation time and preparation personnel; culture medium/solution type and volume; volume of the sub-package; composition, content, manufacturer and lot number of each composition; pH value (initial and final); complementing means, time and temperature of sterilizing measures etc. should be recorded. Keep record of the observation result, data and calculations in situ, and identify the records according to the requirements of specified tasks.

If the lab records need to be modified, two lines should be written on the original records, and don't erase the original records. Then the modified records should be written near the original records with the mender's stamper or signature or abbreviated signature.

2.0 MEASUREMENT/DATA ACQUISITION

2.1 Sampling process design

2.1.1 Test condition preparation

The technical director will take charge in dividing the staff into the sampling group and the test group and assigning task for each team. She is responsible for preparing the related regulations, standards and operating instructions according to the testing task. To determine the instruments, apparatus and the environmental conditions, and purchase and check the chemical reagents and consumables needed for experiment is also one of her responsibilities. Preparation of such stuff as the blank records, sampling bottles, sample labels, sampling facilities, and sample handling reagents and so on is one of her responsibilities as well.

Preparation of the environmental conditions: pressure-washed the feed tank, treated tank, the control tank and all the pipes with tap water, dried and swept before starting testing procedures to remove residual debris, organisms and other materials. Check water source, power supply to be in good condition. Finish the tank intake preparation one day before formal experiment, and the water static and aeration preparation. Check the technical parameters of the treatment device and get ready for the verification testing.

Organisms ≥50 μm and 10 ~ 50 μm which consist of at least 5 species from at least 3 different phyla/divisions will be cultured in certain concentrations, as well as *Escherichia coli*. Prepare the required concentration of organisms before starting each test cycle; and the test is performed until the organisms in the feed tank are well-mixed.

2.1.2 Testing procedures

2.1.2.1 PACT marine™ BWMS installation and commissioning

Installation and commissioning of PACT marine™ BWMS is performed in accordance with the system's Operation and Maintenance Manual as provided to SHOU-BWDL by the vendor. Any deviations made from the Operation and Maintenance Manual will be documented and reported by the SHOU-BWDL TF operation manager. All mechanical, electrical, and plumbing connections should be ensured. The safety of the subject BWMS is evaluated during the installation and commissioning phase and generally throughout the entire test period. Tests will only proceed if the SHOU-BWDL TF operation manager is certain that the PACT marine™ BWMS poses no obvious risk to SHOU-BWDL personnel, the SHOU-BWDL land-based TF and the surrounding environment.

2.1.2.2 Verification testing

Land-based test cycles will be conducted in accordance with IMO G8. A completed test cycle will include all of the following criteria:

- *Challenge water augmentation*: the natural seawater is pumped into the feed tank, with addition of organisms, bacteria etc. to meet the requirements of biological, physical and chemical water quality stated in IMO G8.
- *Ballasting operation on treatment tracks*: the test water contained in the feed tank will be

pumped into the PACT marine™ BWMS, with the treatment of filtration combined with UV irradiation, after which the treated water flows to the treated tank for storage.

- *Ballasting operation on control tracks*: the test water contained in the feed tank is directly pumped into the control tank without any additional treatment for storage.
- *Storage*: the treated water will be stored for 5 days in the treated tank, and the control water will be stored for 5 days in the control tank at the same time. In this moment, keep the tanks out of light. Wash and dry the feed tank and pipes during this storage period.
- *De-ballasting operation on treatment tracks*: after 5-day storage, the treated water will be re-treated by UV irradiation again without filtration and then discharged directly.
- *De-ballasting operation on control tracks*: after 5-day storage, the control water will be discharged directly.
- Wash and dry the control tank, treated tank as well as pipelines to prepare next test cycle.

2.1.3 Sampling points arrangement and sampling facility

2.1.3.1 Sampling points arrangement

The test water in the feed tank will be pumped into the pipelines on the treatment tracks, and samples for influent water on the treatment tracks will be collected from sampling point 01 (SP01). Samples for treated water immediately after treatment will be collected from SP03. Samples for influent water on the control tracks will be collected from SP02. After 5-day storage, samples for the treated water before UV irradiation and after UV irradiation again will be collected from SP04 and SP05, respectively. Samples for the control water will be collected from SP06. The sampling facilities are designed in accordance with IMO G2 (see Section 2.1.3.2). For the isokinetic sampling, a quantitative water sampler is applied to collect the sample. For collecting the bacteria sample, the sterilized sampling bottle is underwater sealing sampling. The arrangement of the sampling points is shown in Figure 10.

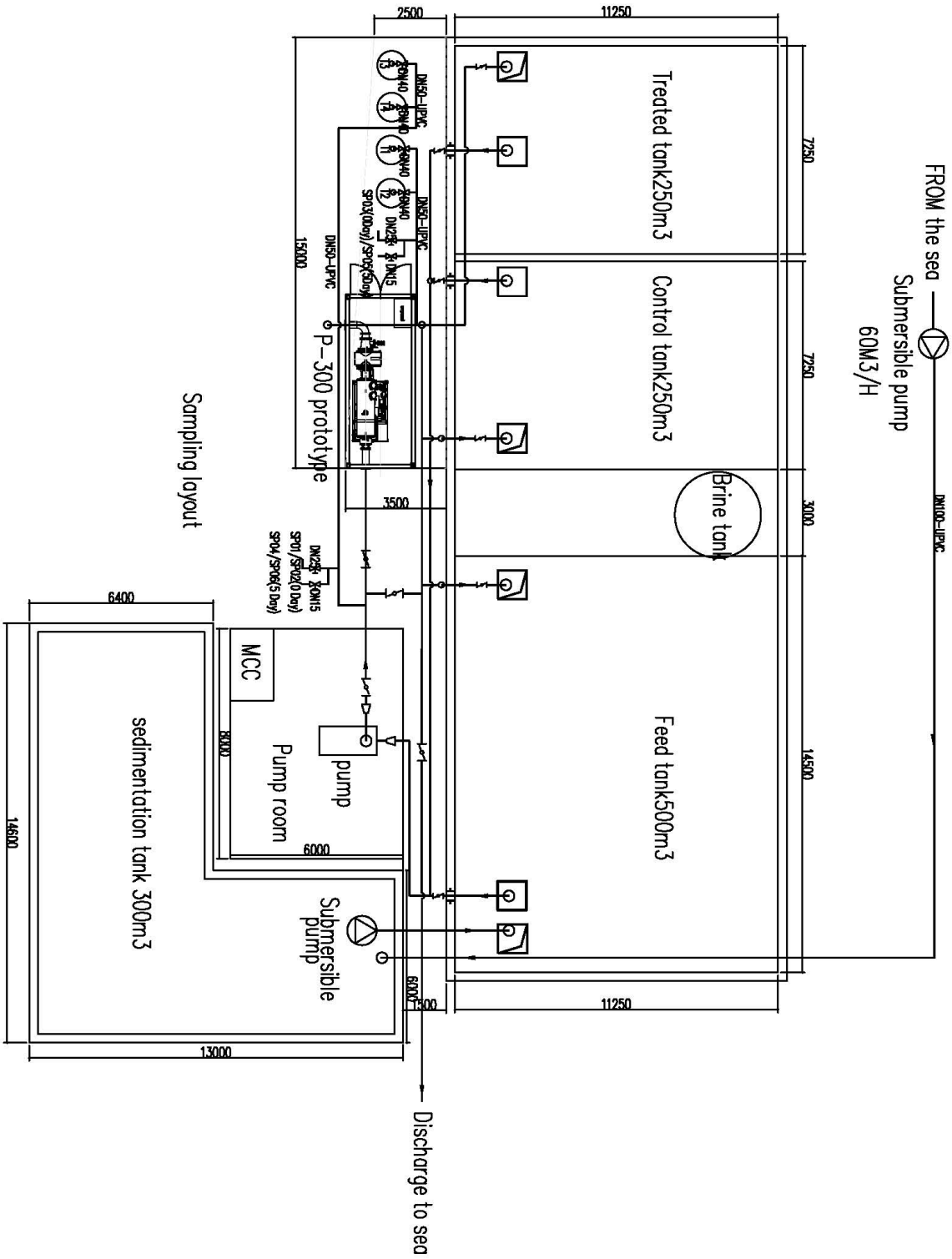


FIGURE 10. Diagrammatical Drawing of Sampling Points

(SP01: influent water on treatment tracks; SP02: influent water on control tracks; SP03: treated water; SP04: treated water after 5-day storage before UV irradiation; SP05: treated water after 5-day storage after UV irradiation; SP06: control water after 5-day storage at discharge)

2.1.3.2 Sampling facility

According to the IMO G2, the sampling facility should be positioned such that a representative sample of ballast water is taken. The sample should be representative of the main stream. The characteristic of the test equipment is taken into account in deciding where the sampling point should be appropriately positioned.

The design of sampling point adopts the isokinetic diameter calculation in accordance with G2, the associated regulations stated in G2 mainly include:

- Through computation fluid dynamics modeling, it has been shown that the isokinetic diameter calculation can provide guidance for sizing determination of sampling points for sampling of organisms.
- Simulations showed that flow transitions from the main stream were best for sampling point diameters between 1.5 and 2.0 times the isokinetic diameter. Ports sized in this range had smooth transitions and pressure profiles that allowed for direct sampling without the need of a pump to induce sample collection. The isokinetic sampling point diameter should therefore be determined generally according to the equation:

$$Diso = Dm\sqrt{Qiso/Qm}$$

  Where D_{iso} and D_m are the diameters of the sampling point opening and the main flow in the discharge line, respectively; and Q_{iso} and Q_m represent the respective volumetric flow rates through the two pipes. It is recommended that sampling point size be based on the combination of maximum sample flow rate and minimum ballast flow rate that yields the largest isokinetic diameter.
- The opening of the sampling pipeline should be chamfered to provide a smooth and gradual transition between the inside and outside pipe diameters.
- The length of the straight sample pipe facing into the flow can vary, but should not usually be less than one diameter of the sampling pipeline. The sampling port should be oriented such that the opening is facing upstream and its lead length is parallel to the direction of flow and concentric to the discharge pipe which may require sampling pipes to be “L” shaped with an upstream facing leg if installed along a straight section of discharge pipe.

The sample points are shown in Figure 11.

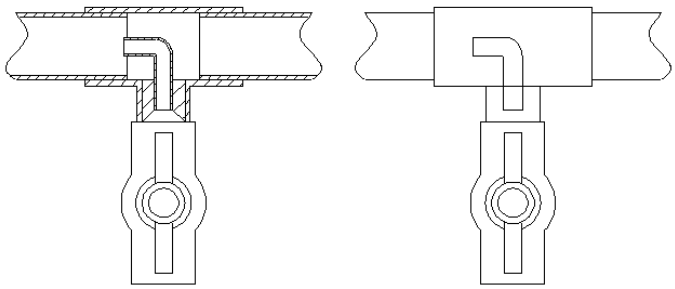


FIGURE 11. Sampling Facility

Since the capacity of ballast pump is 300 m^3/h, taking sampling organisms≥50 μm for example, the diameter and flow of each sampling point is as follows according to IMO G2:

- SP01: sampling volume is 3×20 L, diameter of sampling point is DN25, flow rate is 2.0 m/s, sampling time for single sample is 15 s.
- SP02: sampling volume is 3×20 L, diameter of sampling point is DN25, flow rate is 2.0 m/s, sampling time for single sample is 15 s.
- SP03: sampling volume is 3×1 m^3, diameter of sampling point is DN50, flow rate is 2.0 m/s, sampling time for single sample is 4.5 min.
- SP04: sampling volume is 3×1 m^3, diameter of sampling point is DN50, flow rate is 2.0 m/s, sampling time for single sample is 4.5 min.
- SP05: sampling volume is 3×1 m^3, diameter of sampling point is DN50, flow rate is 2.0 m/s, sampling time for single sample is 4.5 min.
- SP06: sampling volume is 3×1 m^3, diameter of sampling point is DN50, flow rate is 2.0 m/s, sampling time for single sample is 4.5 min.

2.1.3.3 Sampling principle

Samples should be representative and in random with appropriate sample volumes, and the sampling method should be in line with these IMO G2. Samples for the following three categories should be collected respectively:

- Organisms greater than or equal to 50 μm in minimum dimension;
- Organisms greater than or equal to 10 μm and less than 50 μm in minimum dimension;
- *Escherichia coli*, intestinal *enterococci*, *vibrio cholera* (serotypes O1 and O139) and heterotrophic bacteria.

2.1.4 Sampling volumes

Sampling volumes for each sampling point (SP01-SP06) throughout the biological efficacy tests is shown in Table 10.

TABLE 10. Sampling Volumes and Number of Samples Collected from Each Sampling Point

Sampling phase	Influent water during ballasting on treatment tracks		Influent water during ballasting on treatment tracks		Treated water during ballasting		Treated water during de-ballasting (before UV irradiation)		Control water during de-ballasting		Treated water during de-ballasting (after UV irradiation)	
Test items	SP01		SP02		SP03		SP04		SP05		SP06	
	SV	No.	SV	No.	SV	No.	SV	No.	SV	No.	SV	No.
Organisms ≥50 μm	20 L	3	20 L	3	1 m^3	3	1 m^3	3	1 m^3	3	1 m^3	3
Organisms 10~50μm	1 L	3	1 L	3	10 L	3	10 L	3	10 L	3	10 L	3
Bacteria	700 mL	3	700 mL	3	700 mL	3	700 mL	3	700 mL	3	700 mL	3
Water qualities	5 L	3	5 L	3	5 L	3	5 L	3	5 L	3	5 L	3

2.1.5 Sampling phase design

Samples are collected at a sequence of beginning, middle, and end. In cases when the treatment capacity is 300 m^3/h, the total time for one test cycle is 45 min. Table 11 shows the detailed time for sampling arrangement.

TABLE 11. Sampling Phase Arrangement

Sampling point	Sampling time			Remarks
SP01	10 min	20 min	30 min	The previous 5 min will be taken to warm up the system.
SP02	10 min	20 min	30min	The previous 5 min will be taken to warm up the system.
SP03	10 min	20 min	30 min	The previous 5 min will be taken to warm up the system.
SP04	10 min	20 min	30 min	The previous 5 min will be taken to warm up the system.
SP05	10min	20 min	30 min	The previous 5 min will be taken to warm up the system.
SP06	10 min	20 min	30 min	The previous 5 min will be taken to warm up the system.

2.2 Sampling methods

2.2.1 Sample collection

First microbiological samples are collected and then samples for water quality. Table 12 summarizes which sampling equipment is used to collect samples for the individual parameters.

TABLE 12. Sampling Equipment and Containers Used for the Individual Parameters

Parameter	Sampling equipment	Sample container
Organisms ≥50 μm	Directly	Clean HDPE bottle
Organisms ≥10 ~ 50 μm	Directly	Clean HDPE bottle
Heterotrophic bacteria	Directly	Clean glass bottle
E. coli	Directly	Clean glass bottle
Intestinal *Enterococci*	Directly	Clean glass bottle
Vibrio cholerae	Directly	Clean glass bottle
Salinity	Directly	Clean HDPE bottle
Temperature	Directly	Clean HDPE bottle
TSS	Directly	Clean HDPE bottle
POC	Directly	Clean HDPE bottle
DOC	Directly	Clean HDPE bottle
pH	Directly	Clean HDPE bottle
Turbidity	Directly	Clean HDPE bottle
TRO	Directly	Clean brown glass bottle
DO	Directly	Clean brown ground-glass stoppered bottle

The procedures for collecting samples are as follows:

(1) Salinity and temperature are measured in situ with salimeter and thermometer. Water quality parameters including total suspended solids (TSS), dissolved organic carbon (DOC), particulate organic carbon (POC), pH and turbidity will be collected in different sampling time

interval of 5 L in each replicate. Samples are to be stored in dark cool place and delivered to the lab as soon as possible. Total residual oxidant (TRO) is to be collected using a brown bottle with a capacity of 100 mL. The bottle should be tightly closed with a stopper. Dissolved oxygen (DO) is to be collected using a ground-glass stoppered brown bottle with a capacity of 125 mL.

(2) Sampling of organisms 10~50 μm from SP01 and SP02: Organisms with a minimum diameter between 10 and 50 μm are sampled as 3 × 1 L with a 1-L quantitative water sampler. The sampled water is slowly sieved through a plankton net (10 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in one sample bottle.

Sampling of organisms 10~50 μm from SP03 ~ SP06: Organisms with a minimum diameter between 10 and 50 μm are sampled as 3 × 10 L with a 10-L quantitative water sampler. The sampled water is slowly sieved through a plankton net (10 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in one sample bottle.

(3) Sampling of organisms ≥50 μm from SP01 and SP02: Organisms with a minimum diameter larger than 50 μm are sampled as 3 × 20 L. Sampling of organisms ≥50 μm from SP03 ~ SP06. Organisms with a minimum diameter larger than 50 μm are sampled as 3 × 1 m^3. The water is directly collected in one 1m^3 sample bucket and the water is slowly sieved through a plankton net (50 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in one sample bottle. The sieved water is controlled by a flow meter to ensure accurate sampling volume.

(4) Sampling of bacteria from SP01 ~ SP06: Bacterial are sampled as 3 × 700 mL. A clean and sterile glass container will be used when taking the microorganism sample. In order to avoid being polluted, the mouth of the sampling container must be sealed with alcohol cotton, and ignite it when sampling. If there is residual chlorine in the water sample, add sodium thiosulfate which is germ free to the glass container.

2.2.2 Sampling/test system failure response and remedy

Any sampling and testing interruptions or unexpected things occur during sampling should be given a due consideration and causes of them should be found. Report to the technical director in time and keep detailed record of the failure event. If it is resulting from instrument system failure, continue to test by applying another same instrument if necessary. Or else, repair immediately after report to the technical director.

Once the test is interrupted, cut the power supply in accordance with operation procedures.

There are circumstances when external accidents such as power failure, water failure and so on, happen which will affect the testing quality. Therefore, re-sampling and retest are needed when it returns to normal. When it happens that the failure of instruments or apparatus causes the test to breakup. If there is a backup instrument, use it to replace the faulty one and continue the test. If there is only one instrument, and the failure of the instrument will affect the test quality, re-sampling until the instrument returns to normal. The instrument will be handled according to the Procedures for Instrument and Apparatus Management. If something is wrong with the sample, stop the test and report to the technical director. Check the sample, find out the causes and make a suggestion. After being approved, decide how to solve the problem. Re-sampling and re-test are necessary.

Keep record of all abnormities and interruptions occur in the handling process and fill in the sampling/test process and the result abnormalities handling record table. And report to the technical director.

2.3 Sample handling and custody

2.3.1 Sample handling in situ

After sampling organisms 10~50 μm from SP01 and SP02, add 2 mL staining solution to 1-L samples for 15 min and add formalin to fix the samples. After being settled for 24 hours, the supernatant is extracted, and the condensed samples will be added to the specimen bottle with capacity of 60 mL. A constant volume 50-mL sample is analyzed under microscope. After sampling organisms 10~50 μm from SP03 ~ SP06, add 5 drops of staining solution to 60-mL concentrated samples for 15 min and add formalin to fix the samples. A constant volume 50-mL sample is analyzed under microscope.

After sampling organisms≥50 μm from SP01 ~ SP06, add 5 drops of staining solution to 60-mL concentrated samples for 30 min and add formalin to fix the samples. After being settled for 24 hours, the supernatant is extracted, and transfer left organisms to a cone centrifuge tube for sedimentation. Finally, absorb the supernatant and the total numbers of organisms are counted under microscope.

If there is residual chlorine in the water sample, the samples for bacterial analysis are pretreated by adding some sodium thiosulfate (0.1 mL 10% sodium thiosulfate per 120 mL water sample can reduce 15 mg/L residual chlorine).

A quantitative liquid filling device is to be used to drop 1 mL manganese chloride solution and 1 mL alkaline potassium iodide solution immediately after collecting sample. Make sure the sample bottle cap is tight (no air bubbles in the containers after sealed), shake the bottle ups and downs for no less than 20 times, seal the sample and then take the sample to the lab for testing as soon as possible.

2.3.2 Sample preservation

Samples for water quality parameters should be stored in dark. TSS, DOC and POC should be processed within 24 h after sampling and stored in dark. When the samples arrived at the laboratory, they are stored in a cool room. If POC and DOC testing cannot be finished within 24 h, add a small amount of $HgCl_2$ in sample and stored under -20°C for 7 days. TRO, pH, turbidity and DO should be analyzed within 6 hours. Samples for organisms analysis are fixed with formalin in situ, placed in the dark and delivered to the laboratory. Analysis should be finished within 7 days. Samples for TRO and bacteria are packed in a cooler bag (0~5°C) under transport.

After tested, the samples can be store for a long term by being placed in fixing liquid solution and dark place. Add some formalin every three months to avoid samples decay. The bacteria samples should be tested right after transported to the lab since there is no way for long-term storage. Samples for water quality parameters are not suitable for long-term storage after tested. If retesting is necessary, the holding time should be kept within the valid testing period.

2.4 Analytical methods

2.4.1 Organisms ≥50 μm

The samples being filtered and concentrated are identified and analyzed by total count method and counted by kind/species to calculate the organism number (number of organisms in per unit)(GB17378.7-2007):

$$r_B = \frac{N_B}{V}$$

Where:
r_B——density of organisms ≥50 μm in per unit of volume
N_B——number of zooplanktons (ind);
V——volume of filtered water, unit:(m^3).

Detailed information on organisms ≥50 μm determination is provided in Appendix A.

2.4.2 Organisms 10~50 μm

Lightly absorb the supernatant fluid from the pretreated samples using a suction pipe with 10μm bolting silk. After settling down for a few times, the water sample is condensed to a 50ml thimble tube. Shake enough before sampling counting, absorb a certain amount of sample and then release it at the counting chamber covered with cover glass (make sure there are no bubbles remain) and then conduct the microscopic counting (GB17378.7-2007).
Optical microscopic counting (concentrated counting):

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

Where:
C——total amount of samples in per unit volume, unit:(cells/mL)
n——number of samples, unit:(cells);
V_1——the volume of concentrated water sample, unit: ml;
V_2——volume of filtered water, unit:(mL);
V_n——volume of sampling counting, unit:(ml)

Detailed information on organisms ≥10 μm to <50 μm determination is provided in Appendix B.

2.4.3 Bacteria sample analysis

2.4.3.1 Determination of heterotrophic bacteria in water sample

Add 1 mL of tween-80 solution to per 100 mL of bacteria sample solution. Gradient dilution is made by high pressure sterilized seawater. Before the water sample is diluted, shake it with effort to make it mixed sufficiently. 10 mL water sample is sucked by a sterilized suction tube and added to 90 mL sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100 times, 1000 times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. Take 0.1 mL diluted water sample and spread it uniformly on the 2216E culture medium.

Four dilution degrees of each water sample are needed to be prepared and replicate two plates for each dilution degree. Put the plate into a constant temperature culture box (25°C) with its upside down for 7 days. Count the total number of the colonies with a stereomicroscope: (1) Do not count when large lawn appears on the plate; (2) Plate with number of colonies between 30 and 300 is selected; the average number of colonies multiplies the dilution degree (10 times, 100 times or 1000 times) equals to the number of the bacteria in water sample; (3) If there are two kinds of dilution degrees with average number of colonies are between 30 and 300, the ratio of the two numbers determines which one to choose. If the ratio is less than 2, the average of the two is chosen; if more than 2, the colony with less number is chosen; (4) If all the average values of all kinds of degrees of dilution are more than 300, the number of colonies is counted using the average number of colony in the largest degree of dilution (lowest concentration) multiplies the times of dilution; (5) If all the average values of all different degrees of dilutions are all less than 30, the number of colonies is counted using the smallest degree of dilution (highest concentration) multiplies the times of dilution; (6) If there are no colonies in all different degrees of dilution, and no inhibitor is tested, then report less than 1 multiplies the lowest diluted times.

Detailed information on heterotrophic bacteria determination is provided in Appendix C.

2.4.3.2 Determination of *Escherichia coli* in water sample

The samples should be tested by multi-tube fermentation method immediately after they are transported to the lab in cool conditions in accordance with the specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring GB17378.7-2007 and Standard examination methods for drinking water—indicator microbes GB/T5750.12-2006.

Shake the water sample over 25 times at least before testing or diluting to make the water sample well mixed. 10 mL water sample is sucked by a sterilized suction tube and added to 90 mL sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100 times, 1000 times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. A 10 mL of the original water sample is inoculated to the 10ml two-fold lactose peptone culture medium; a 1ml water sample is inoculated to the 10 mL unblended lactose peptone culture medium.

Furthermore, take 1 mL 10^{-1}, 10^{-2}, 10^{-3} diluted water sample to the unblended lactose peptone culture medium, five tubes of each degree of diluted water sample are inoculated. Put the inoculation tube into a culture box with a temperature of 36±1°C for 24±2 hours. If no bubble or no acid is produced in the lactose peptone culture pipe, it indicates the *Escherichia coli* to be negative. If there is gas or acid produced, follow the steps bellows: inoculate the water sample in the fermentation tube to the eosin methylene blue agar plate, and put it in the 36±1°C culture box for 18-24 h. Observe the appearance of the colonies, and choose the kind of colony which gets the specific features (dark purple black, metallic luster; purple black, no or little metallic luster; light purple red, dark in the center) for Gram staining, microscopic test and verification test. If the water sample is tested to be Gram negative spore less bacterium, inoculate to the lactose peptone culture liquid at the same time and put it into the culture box with a temperature of 36±1°C for 24±2 h, if there are gas and acid produced, it is a proof of the existence of the *Escherichia coli*.

Test of the *Escherichia coli* in the tube which has fermentation phenomenon and gas or acid in it.

A metal inoculation loop being sterilized by burning or the sterile cotton swab is used to inoculate the liquid in the tube mention above to the EC-MUG tube. Put the tube which has been inoculated into the culture box with a temperature of 44.5±0.5°C for 24±2h. The EC-MUG tube is radiated in the dark by a 6 W power UV lamp with a wavelength of 366 nm, if blue fluorescent light is observed; it shows that there is *Escherichia coli* in the water sample. Count the number of the positive EC-MUG tubes, refer to the most probable number (MPN) table for the matched most probable number of the *Escherichia coli*, reported the result of the number of *Escherichia coli* in the unit of MPN/100 mL.

Detailed information on *Escherichia coli* determination is provided in Appendix D.

2.4.3.3 Determination of Intestinal *Enterococci* in water sample

The samples should be tested immediately after they are collected on board in accordance with the standards stipulated in water quality- detection and enumeration of the intestinal *enterococci* ISO 7899.2-2000.

Shake the water sample over 25 times at least before testing to make the water sample well mixed. Connect the sterilized filter device to the Buchner flask, put the membrane at the bottom of the filter with a germfree tweezer, and certain amount of water sample is sucked to the filter, and be sucked and filtered by the vacuum pump. After all the water sample liquid passes through the membrane, clean the edges of the filter with 20 to 30 mL normal saline for twice at least. Then, turn off the vacuum pump and turn on the filter, and take the filtered membrane by a germfree tweezer onto the surface of the mEI agar culture medium- Slanetz and Bartley medium (membrane intestinal *enterococci* culture medium). Ensure that no bubbles in the middle of the membrane and the medium. Put the plate upside down in temperature of 36 ± 2°C for 44 ± 4 h. After the culture time is over, all the colonies which is red, nut brown or pink, no matter in the middle or full over the plate are all typical. If there are typical colonies formed, transfer the membrane and the colonies using the germfree sweezer to the Bile Esculin Azide Agar plate which has been preheated to 44°C, culturing for 2 h at 44 ± 0.5°C, then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as intestinal *enterococci* (note: counting when the colonies are uneven distributed or bulge will affect the identification of the positive colonies. The color will diffuse to the colonies nearby). Count the membranes which are proved to be intestinal *enterococci* colony, cfu/100 mL.

Detailed information on intestinal *enterococci* determination is provided in Appendix E.

2.4.3.4 Determination of *Vibrio cholerae* in water sample

According to "Diagnosis Standard for *Vibrio cholerae* (WS 289-2008)", water samples are inoculated in basic peptone water medium. Place the enrichment medium with samples into incubator for enlargement cultivation at 37°C for 6-8 h. Then the strong and weak nutrient mediums are inoculated, they are used to isolated culture and made cultivated substance morphology observation. *Vibrio cholera* in different isolation mediums presents different characteristics. Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

Detailed information on *Vibrio cholerae* determination is provided in Appendix F.

2.4.4 Water quality parameters

2.4.4.1 pH

pH is measured using a pH meter. Detailed information on pH determination is provided in Appendix G.

2.4.4.2 Turbidity

Turbidity is measured using a turbimeter. Turbidity is reported in °. Detailed information on turbidity determination is provided in Appendix H.

2.4.4.3 Total suspended solids (TSS)

The sample is filtered through a filter (0.45 μm) on board, and the filtered membrane is stored at 4°C until analysis. TSS is measured at BWDL in accordance to National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.4) - Part 4: Seawater analysis. Detailed information on TSS determination is provided in Appendix I.

2.4.4.4 Particulate organic carbon (POC)

POC is determined based on spectrophotometry method. According to "Chinese Gulf ecosystem observation method (2005)", carbon is wet oxidized by acidic dichromate; the decrease of the extinction value of the yellow dichromate solution may indicate the quantum of oxidized carbon. Detailed information on POC determination is provided in Appendix J.

2.4.4.5 Dissolved organic carbon (DOC)

The collected sample is filtered by the Whatman GF/C fiber glass membrane and then tested by a TOC analyzer (GB17378.4-2007). Detailed information on DOC determination is provided in Appendix K.

2.4.4.6 Total residual oxidant (TRO)

Determination of total residual oxidants in waters using N,N-diethyl-p-phenylenediamine (DPD) Spectrophotometry(HJ 586-2010). Detailed information on TRO determination is provided in Appendix L.

2.4.4.7 Dissolved oxygen (DO)

Determination of dissolved oxygen by iodimetry: A dissolved oxygen burette is applied to measure DO (GB17378.4-2007). Detailed information on DO determination is provided in Appendix M.

2.4.4.8 Salinity

Salinity is measured *in situ* using a calibrated salimeter. Salinity is reported in PSU. Detailed information on salinity determination is provided in Appendix N.

2.4.4.9 Temperature

Temperature is measured in situ using a calibrated thermometer. Temperature is reported in °C. Detailed information on temperature determination is provided in Appendix O.

2.5 Quality control

Four data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data obtained in the project. These are precision, operator bias, comparability and representativeness, and their protocols are described.

2.5.1 Precision

Precision refer to the measure of agreement among repeated measurements of the same property under identical or substantially similar conditions; calculated as either the range or as the standard deviation. It may also be expressed as a percentage of the mean of the measurements, such as relative range or relative standard deviation (coefficient of variation).

For samples of organisms ≥50 μm and ≥10 to <50 μm, within sample precision is measured by analyzing at least two slides from every sample collected. Precision is quantified by calculating the coefficient of variation (CV) among the subsamples analyzed for each sample using the following equation:

$$\%CV = \frac{SD}{mean} \times 100\%$$

Where, SD = standard deviation among subsamples; mean = mean live organism density among subsamples. The acceptable analytical precision is ≤30% for critical parameters.

With respect to samples involving water quality and bacteria, analyzing at least 10 percent of total samples in duplicate and calculating the Relative Percent Difference (RPD) as determined by the following equation evaluate precision:

$$RPD = \frac{|x_1 - x_2|}{\frac{x_1 + x_2}{2}} \times 100\%$$

Where, x_1 = sample; x_2 = duplicate sample. The acceptable analytical precision is ≤20% for critical parameters.

2.5.2 Operator bias

Bias refers to the systematic or persistent distortion of a measurement process that causes errors in one direction.

Operator bias relative to organisms ≥50 μm are evaluated for every treatment discharge sample collected at the shipboard testing. In this situation, one out of every ten slides analyzed by the primary taxonomist is also analyzed by a second, suitably-qualified zooplankton taxonomist. The duplicate analysis is conducted such that the second operator does not know the results of the first operator's analysis. Additionally, one out of every ten influent or control discharge samples is analyzed by a second zooplankton taxonomist. In the event that there are less than ten total control samples collected during a treatment technology performance evaluation, at least one influent or discharge control sample is evaluated, with one slide analyzed in duplicate.

Operator bias relative to samples of organisms ≥10 to < 50 μm testing are evaluated using at least two treated discharge samples per set of three test trials and at least one influent or control discharge sample per set of three test trials. In this situation, for every sample analyzed by the

primary taxonomist that requires evaluation, a second, suitably qualified taxonomist simultaneously analyzes the same sample. The analysis is conducted such that the second operator does not know the results of the primary operator's analysis, and vice versa.

BWDL evaluates operator bias for samples of bacteria by having a second, suitably-qualified operator count at least 10 percent of all experimental chambers. Analysis occurs immediately following analysis by the first operator and is carried out in a manner such that the second operator does not know the results of the first operator's analysis.

The BWDL performance measurement for these sample types is relative percent difference (RPD). The acceptable RPD is <20% for critical parameters.

2.5.3 Comparability

One control test run will be conducted for each of test cycle as a reference and to identify any changes in critical parameters not caused by the equipment itself.

2.5.4 Representativeness

Representativeness is a qualitative measure of the degree to which data accurately and precisely represents a characteristic of a population parameter at a sampling point or for a process condition or environmental condition. The representativeness will be achieved through (1) all samples will be taken in triplicates; (2) complete mixing will be ensured before any withdrawal of samples or *in-situ* measurement; (3) one control will be conducted for each of the test cycle to identify any changes in critical parameters not caused by the equipment itself.

2.6 Instrument/equipment testing, inspection, and maintenance

The instrument manager is responsible for compiling the Check List of the instruments, and establishes the file of instruments and identifies the instruments with related labels. The operator of instruments should be an authorized staff or one with vocational test staff certificate. All operators should be approved by the lab to carry out the operation. Use of equipment should be strictly in accordance with operating procedures. The staff is asked to operate the instrument as trained to be in order not to get the invalid test result. The user of the instruments should check the status an environment condition of the instrument (including whether it is in valid period, need maintenance or not, if stabilized or not) before and after use. And fill the Use Record of the Instrument.

If there is abnormal phenomenon (overloading, wrong operation, questionable result displayed) for the equipment, the user should stop the operation and stick a red mark on it. Separate the abnormal instrument to avoid misuse. If the instrument falls out the direct control of the lab for example: removed to other places, sent for repair or calibration, after the instrument is back, the instrument attendant should check the function and the calibration status of the instrument and recovered to use until the results displayed are satisfying. The instrument manager takes charge of checking the instruments to prevent the instruments from damaging and losing. Make an inventory of the instruments annually. If there is damage or lose of instrument, repair or handle in accordance with the Control Procedures for Nonconsistant Test Work.

A specified worker is appointed for the maintenance of the instrument in use. Power on once per month at least (1 - 2 h) to check if the instrument is normal and keep record. The instrument manager is responsible for organizing the instrument user to make the routine maintenance

plan, and to form the Routine Maintenance Table of Instruments. The instrument user makes the maintenance of the instrument to comply with the items and periodic times in the table and keep the record meanwhile.

2.7 Instrument calibration and frequency

Instruments need to be verified and calibrated by legal metrology verification service agency. The instruments are delivered by the lab synthesizer according to the Quantity Traceability Procedures, aiming to get the qualified certificate. The instrument attendant performs the periodic calibration of the instrument. If the correction factors are obtained after instrument calibration, the instrument attendant is responsible for updating of all the backups and the correcting of related data. The frequency for calibration is once per 12 months, once per 6 months for special instrument. Refer to Table 13 for the calibration requirements of the instruments that will be used in the test.

TABLE 13. Calibration Requirements of the Testing Instruments

No.	Parameters	Equipment Name	Model	Measuring range	J expand	Frequency of Verification Calibration Requirement
1	Heterotrophic bacteria	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
2	*Escherichia coli*	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
3	*Vibrio cholerae*	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
4	Intestinal *enterococci*	LDZX model vertical pressure steam sterilizing pot	LDZX-75KBS	(50~126)°C	U=0.5°C, (k=2) ± 0.2°C	Annually
		Model DK electric-heated constant temperature water bath kettle	DK-S26	(RT+5~99)°C	U=0.3°C, (k=2) ± 0.3°C	Annually
5	Phytoplankton (10-50 μm)	optical microscope	S8APO	(40~1600)x	±5%	Annually
6	Zooplankton (≥50 μm)	optical microscope	DM500	(40~1600)x	±5%	Annually
		stereomicroscope	PXS	(10~80)x	±5%	Annually
7	TSS	electronic balance	AL104/01	(0.0001~110) g	I grade	Annually
8	Temperature	thermometer	(0~40)°C	(0~40)°C/0.2°C	0.62°C	Annually
9	POC	ultra violet spectrophotometer	UV-2000 model	(190~2600) nm	IV grade	Annually
10	Salinity	salimeter	SYA2-2	2-42 PSU	U=0.0038, (k=2)	Annually
11	DOC	TOC analyzer	TOC-V CPH/CPN	(0.01~1000)μg/L	±0.5 mg/L C	Annually
12	pH	pH meter	FE20	0-14	0.01	Annually
13	DO	Burette	25 mL (Ex)	0 -25 mL	B grade	Annually
14	Turbidity	Turbidimeter	WGZ-3	0-1000 NTU	3.90%	Annually
15	TRO	ultra violet spectrophotometer	UV-2000 model	(190~2600) nm	IV grade	Annually

2.8 Quality management plan

As the testing organization, the Ballast Water Detecting Lab of Shanghai Ocean University will carry out the project quality management in compliance with the Quality Management Plan (QMP) and takes part in the comparison of the testing results with those obtained by competent labs specialized in the same testing field, and participates in the proficiency testing program organized by authorized organization according to the lab file which is called the Procedures for Testing Result Quality Control.

Retest of the samples in the retention time, retest the same sample by the same method or different method, and retest the same sample using the same instrument or different instrument to assure the quality of the test result. Keep the sensitivity, accuracy, deviation allowance range, precision of the parameters; ensure the reliability and integrity of the data.

Enhance the quality awareness of the test personnel; make a clear division of quality responsibilities. The test undertaking organization should be supervised by the entrusted organization and the technical supervision organization. The test undertaking organization should take the quality control procedures in the test process into the quality operation system and make the quality plan to comply the quality system and requirements of the testing project.

2.8.1 Quality management of field sampling

Assure the quality of field sampling and analysis. Prepare the procedures for conducting the sampling and avoid the samples being polluted. Keep away from the interference of the instruments. Select sampling facilities, sample bottles appropriate for the testing items. Take antipollution measures for accessories in testing place; minimize the influence of the interface concentration. The pretreatment of samples should be completed in situ right after the samples are collected, and then add some stabilizer and store in low temperature. Items which are susceptible for microbes' activities or change fast with time should be finished testing within the stipulated time.

Indicators like salinity, temperature should be measured in field. During the field test, turn on the instrument and leave it to be warmed-up until the instrument reading and the flow of the ballast water management system become stabilized, and then wash the sample bottle for two times with a little of water sample, afterwards, fill the bottle up with sample, the probe of instrument in the sample bottle, get the reading after the instrument is stabilized. When collecting the suspended solid water sample, wash the sample bottle for two times with a little of water sample, and then fill the sample bottle to the fully slowly. Ensure the water samples are stored in cool and shady place and transported to the lab. Ensure the water sample be filtered within 24 h. The POC samples collected simultaneous are divided in the lab; try to use the ground glass sample bottle to collect the samples in order to avoid the absorption of C of plastic products. Before use, all the glass containers should be immersed in the Sulfuric acid and potassium dichromate lotion for 24 - 28 h; then be rinsed with tap water and washed again with de-carbonized water, and the de-carbonized water should be prepared in advance. When collection samples in situ, wash the sample bottle with a little of water sample first and then collect the samples and refrigerated transport the sample to the lab.

Collect 10 - 50 μm viable organisms sample by method of sediment and concentration counting of certain volume of water sample. There is no need to rinse the sample bottle when collect the samples, collect certain amount of well mixed water samples. Water sample of viable organisms greater than 50 μm is prepared by collecting the organisms filtered by a 50 μm screen organism net and count the total number. Ensure the organism net is clean and tried before collecting.

Viable organism staining agent should be prepared temporarily (to avoid the failure of staining agent as time goes) before sampling. Add quantitative staining agent to the collected viable organism sample for enough time and then add formalin to fix the sample. Right after the viable organism samples are collected, transport them to the lab (protect them from light and vibration) and analysis as soon as possible. The microbe sampling and test personnel should be trained and be equipped with basic knowledge of microbes. When conduct the microbe testing, all the containers should be sterilized. Prepare a set of trip blank and field blank during each sampling process. One regent blank should be prepared in each batch of samples or ten samples. Keep record of all the original data of the initial dilution water samples for review. Each dilution degree of water sample should replicate.

2.8.2 Quality management of lab sample analysis

2.8.2.1 Quality management of chemical reagents

Chemical reagents used in lab sample analysis should be prepared to solutions in accordance with prescribed conditions. The solutions should be stored in right conditions and used within the prescribed period. The self-prepared solutions are allowed to use unless they are calibrated to be qualified with the guarantee value of national standard solution. The blank value of reagent should be in the same level with the analysis detection limits. If the value is too far over the detection limits, the causes need to be found. And main agents are purified which have great agent blank value or change the reagents (use a new batch number of agents or agents produced by other manufacturers). And all the regents should be checked before use. In the cases when the blank value is hard to be lowered, add appropriate amount of reagent. During analyzing, parallel test the analysis blank and monitor the variation of the blank value.

2.8.2.2 Quality management of containers

Make a clear understanding of requirements for the materials used in containers, select the right material. The characteristics of container material should have the least pollution to the water sample and be easy to clean. And it should be inertia to the chemical activity and biological activity to protect the water sample from reacting with the container to the maximum extent. The capacity of dealing with temperature fluctuation, resistance to rupture, sealing property, capacity of reopening, volume, shape, mass and possibility for reuse of the sample storage containers should be taken consideration when selecting the containers.

For most samples which include inorganic compositions, containers which are made of polyethylene, polytetrafluoroethylene or eater polymer are chosen to use; for the storage of samples for determining and analyzing the conductivity and pH in water, containers which are made of high density polyethylene are used; for the storage of organic chemical and organism samples, glass containers are used. The containers should be cleaned in the right way; the compositions of the detergent should not include the substance to be tested. The new container should be cleaned thoroughly; the substances to be tested determine which detergent to choose.

For general use, taps water and detergent are used to clean dust and packaging matter, then immersed in the chromic acid and sulfuric acid detergent, and at last rinsed with stilled water. For those used containers, there are usually grease, heavy metal and residents in the bottom and wall of the container, there once they are reused, and they must be cleaned before being used. For those glass containers with stoppers, the ground part is often with digestions and absorptions. Polyethylene is susceptible to absorb oil or grease, heavy metal, sediments and organisms and it is hard to clean. So, much attention should be paid when cleaning the containers made of polyethylene. Before the container made of polyethylene is used, clean with

1 mol/L hydrochloric acid solution and immersed in the (1+3) nitric acid solution for a long time. Before the sample bottle used for storage and environmental parameters analysis is used, clean it with nitric acid solution, and then rinse with stilled water to remove the heavy metal and chromate residual. If the organic composition to be determined is tested after extraction, the glass bottle may be cleaned with extraction detergent.

2.8.2.3 Quality management of instrument

The analysis instrument for testing should be in compliance with the stipulations of the Specification for Ocean Monitor GB/T 17378. Instruments are checked and calibrated by specified personnel in regular times. The instruments should be cleaned with stilled water after being used and immersed in the protection liquid to avoid the residual of samples and corrosion of the instruments. Or maintain the instruments according to the instrument operation manual to keep off of measurement error next time, and conduct the instrument interval check as necessary.

2.8.2.4 Quality management of environmental parameters

After POC samples are collected, use fiber glass membrane with 47 mm diameter and 0.45 μm diameter and the standard micropore filter to treat the samples. The membrane must be burned in the 450-500°C muffle for 24 h wrapped in aluminium foil to remove the oxidizing substances (the burning temperature should not exceed 500°C, or else, the filtration characterization of the membrane will change).

When the TSS is filtered, clip the membrane with a stainless steel tweezer for fear of pollution. Prevent the seawater from flowing backward and then damage the vacuum pump. And drain out the wastewater in time. Keep the ambient tidy when drying the sample.

2.8.2.5 Quality management of lab test methods

The lab can undertake the test task on condition that it is accredited the Metrology certificate. Test method is selected mainly based on the precision, accuracy and detection limits of method, to give due consideration of factors such as cost, instrument condition and test cycles and the skill level of personnel. The test methods used should be verified by standard novelty search.

2.9 Data collection requirements

The technical director summarizes the results obtained both from filed work and lab test for shipboard test and organizes the data acquisition and statistics. Prior to data statistics, the test personnel should check the test data first. Check if the original data is integrated and if it meets the requirement, if the calculation and conversion of data is right. Mutual correction is preferred by test personnel after the test data is checked by the test personnel. The reviewer should carry out the review in conformance with the standards, procedures, norms and enforcement rules, and if calculation is required, the calculation for mulas and the calculation process should be checked. Check whether the calculations, the rounding off and the conversion are right. The reviewer should review the original data thoroughly at the time of checking the test reports for the reliability and the matching of the data. The data verified to be right is collected and summarized by the technical director.

2.10 Data management

The sampling data and data determined in field should all be record in waterproof table or to create the electronic document right after the samples are taken in field. The management of the electronic data is in accordance with the Procedures for Computer Management.

2.10.1 Data record

To make the record meet the standard and ensure that sufficient information is collected, the lab adopts the uniformed and approved form of record table. The sampling records should be prepared with pen or ball-pen. The handwriting should be clear. And the record should be verified and integrated. There should be date of record, signature of recorded person and the record number on the record, and the technical record should include the signatures of test personnel and reviewers. The technical record should include technical parameters. All the technical parameters, data, observation results and calculations should be kept being recorded in time, with no replenish.

If the sampling records need to be modified, two lines should be written on the original records, which should be made out. Then the modified records should be written on the blank on the top right of the original records with the mender's stamper or signature or abbreviative signature. All the records should be collected, filed and preserved.

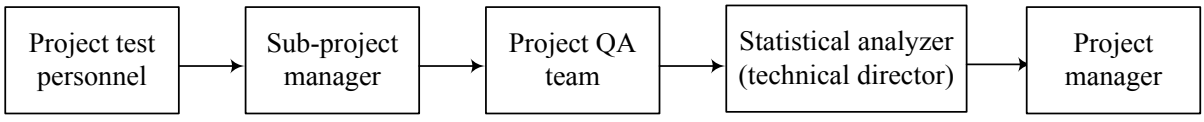
2.10.2 Data confirmation

The test personnel conduct the test in accordance with the requirements and standards related. The quality supervisor takes necessary actions to oversight the test. After the test is finished, the technical director need to sign for confirmation.

2.10.3 Data conversion

The quantity of the plankton is counted and converted to the uniform unit required by the project by statistical analysis. Therefore, organism samples with particle sizes between 10 μm and 50 μm are converted into cells/mL; particle sizes greater than 50 μm are converted to cells/m^3; the bacteria samples are converted to cfu/100 mL.

2.10.4 Data delivery



2.10.5 Data analysis

The original data record is collected by related test personnel, and the test personnel will calculate the final test results according to the conversion method of the parameters and the corresponding curve. The calculation process of data is included in the original record. Take the assessment for the uncertainty occurs in the testing process based on the stipulations in the standards for testing all parameter and determine the significant digits for values. At last, the technical director analyzes the removal rate of organisms with different particulate diameters at each steps of ballast water management, and calculates the removal effect. The rounding off method of specific values is as follows:

1) Refer to *The Rule of Data Revising* (GB 8170-87) for rounding off the values

The rule for rounding off of the numerical values is a round 5 (the digit of the tested valid number is determined): when the rounding off number of the measured value is less or equal to 4, then rounding down; if the rounding off number in the measured value is more than or equal to 6, then rounding up; if the rounding off number in the measured value is equal to 5, rounding up if the mantissa rounded up number is even number, and rounding down if the mantissa rounded up number is odd number. The measured values are rounding off by this way.

In calculating and reading of data, the digits of data might be more than prescribed, for example, the digits of data calculated in calculator may be 7, and when weighed on the analytical balance, only 5 digits of data is obtained, so it is necessary to rounding off the redundant digits. The process for cutting the redundant digits or digit is called the rounding off process, and it is in accordance with the rules of Four Rounding Down and Five Rounding up.

2) Data calculation rules

The data calculation rules are determined by the law of error transmission.

Plus-minus method: transmission of the absolute errors of the measured values. The absolute error of the max absolute error of measured values determines the uncertainty of the analysis result. Therefore, the retention of the significant digit of the summed value of several measured values should base on the number which has the least digits after the decimal point.

Multiply-division method: transmission of the relative errors of the measured values. The relative error of the result should be in accommodation with the value with the max relative error. Therefore, rounding off of the values should be in accordance with the least significant digits.

Scale values of the volumetric containers used for titrimetric analysis (burette, volumetric flask, and pipette) are all with four significant digits. So the number of significant digits of the test data result is four.

3) Formula for calculating removal rate

Removal rate = (density before treated – density after treated) × 100%/density before treated

2.10.6 Data storage and retrieval

All the test data record should be kept by the data administrator. The preservation time of the copies of the original test record, test reports is five years and the data administrator takes charge of the safe custody of the files and records. The records should not be let out or loaned to people unrelated and the customer's business secret should be kept.

The internal staff should go through procedures for loan or copy of the documents, and he or she should fill in the registration table. For external staff who wants to loan or retrieve the records, he or she should be approved by technical director, after the technical director give approval, he or she can go through the loan procedures and fill in the registration table. Read on site, no taking away. The user or keeper of the records should comply with the procedures for keeping the secret and proprietary of custom, do not copy without permission and forbid revealing.

3.0 ASSESSMENT AND RESPONSE ACTIONS

3.1 Assessment and response actions

Project supervisor of the shipboard BWMS testing project is responsible for conducting a continuous improvement actions based on the quality policy, quality objectives, approved result, data analysis, data correction, preventive measures and management review including analyzing and assessing the status; looking for and finding the aspects needed to be improved (looking for the improvement opportunity); ensuring improvement aims; establishing improvement scheme and reviewing the scheme, then selecting the optimal one; implementing the responsibilities and related resources, and putting forward the improvement scheme; monitoring and measuring the implementation situation to make sure whether the it is effectively implemented; formally taking the effective measures; the corrective and preventive actions should be taken into the plan and the management of daily improvement activities.

To determine the causes of discrepancies and look for the improvement chance by way of internal approval, management review, custom feedback, ability verification or other way of data analysis of quality control result. If preventive measures are taken, supervise and monitor the implementation of them, to minimize the possibility of nonconformities and look for improvement chance. Conduct the assessment in accordance with the lab's improvement control procedures, correction measures procedures, preventive measures procedures, test result quality control procedures and the management review control procedures and take the emergency response measures.

3.2 Testing report

The technical director of the testing organization submits the test reports, and the quality manager of the testing organization submits the uncertainty report to the quality management team of shipboard test for ballast water management system. The supervisor of the testing organization briefly summarizes the results of related parameters and proposes a new project quality assurance plan to the supervisor of the entrusted organization for summary and renewal. The test reports of each item and the test results should be precise, clear, and objective and conducted in compliance with the test methodology.

Each test reports should include at least information as follows: test designing, identification of the methodology, status description of the tested material and cleared label, the acceptance date and the test date, the test result, the test report approver or equivalent mark; if the test result need to be explained, there should be announcement about the test method deviations and evaluation uncertainty included in the test report. In the cases when the testing results provided by a subcontract party are included in the testing report, those results should be marked clearly. The subcontract party should report the results in the way of paper edition or electronic edition.

4.0 DATA VALIDATION AND USABILITY

4.1 Data review, verification and validation

Check and review all the data from field determination and lab test and verify the integrity, continuity, validity of the data, and check whether the items meet the requirements. When the data results are in consistent with the quality control data of ballast water management system and the data quality achieves the objectives of this project, then the ballast water management system is acceptable.

4.2 Verification and validation methods

The review, verification and validation of date should be performed to ensure the data meets the criteria. The verification and validation methods include self-assessment, taking part in the reconciliation activities with other labs organized by authorized parties and the ability verification plan. The authorized signatory verifies the quality control data by statistical technology annually and makes the verification reports, then input the management review. If the quality control results are not satisfying or unstable, look for the causes for problems and take actions in accordance with the lab's Corrective Measures Procedures, Discrepancy Test Work Control Procedures, and Preventive Measures Procedures.

The data validation includes all the task plans of the ballast water management system test except the data verification confirmation, including the quality control result assessment for determination of field sampling data, assessment for determination of lab parameters, discrepancy analysis of sample storage and pretreated, the sample test limitation time range verification, the traceability of methodology for test reagents and test standards, verification of the analysis sensitivity in conformance with QAPP, deviation analysis of sampling and analysis with requirements of QAPP, the verification of calculated results, to ensure that QAPP includes relevant information on all the parameters and samples.

4.3 Reconciliation with test data quality objectives

Data generated in this project is analyzed and reconciliated with the data quality and project requirements in accordance with the guidelines for approval of ballast water management systems (G8) and discharge requirements of ballast water (D-2 standard). The data meet the requirements of the project and the D-2 standard, and achieve the treatment effectiveness of ballast water management systems and the data related documents will be applied to the authorized organization as appropriate.

5.0 REFERENCES

IMO (2004). International Convention for the Control and Management of Ships Ballast Water and Sediments. As adopted by consensus at a Diplomatic Conference at IMO, London, England, February 13 2004.

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GB 17378.7 (2007). The National Standard of the Specification for Marine Monitoring-Part 7: Ecological Survey for Offshore Pollution and Biological Monitoring.

GB/T 5750.12 (2006). Standard Examination Methods for Drinking Water-Microbiological parameters.

ISO 7899.2 (2000).Water quality- detection and enumeration of the intestinal *enterococci*-Part 2: Filter membrane method.

GB 17378.4 (2007). The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis.

Chinese Gulf Ecosystem Observation Method (2005). China Environmental Science Press.

Diagnostic Criteria for Cholera. WS 289-2008.

Water quality-free chlorine and total chlorine determination: N,N-diethyl-p-phenylenediamine (DPD) Spectrophotometry (HJ 586-2010).

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End

6.0 APPENDICES

Appendix A Standard Operating Procedure for Organisms ≥50 µm Sample Analysis

1. Scope and Application

This method is suitable for analyzing viable organisms greater than 50 micrometers in minimum dimension. Here we describe a method using a Janus green B staining solution to tag viable cells.

2. METHOD OVERVIEW

Organisms will be enumerated under dissecting microscopes (nominally 10x-80x) to determine concentrations of live and dead organisms ≥50µm in minimum dimension. The samples will be collected in zooplankton nets constructed of mesh with an open square 35µm on each side (50µm on diagonal). The method involves microscopic examination of the living or dead organisms ≥50µm in minimum dimension by the staining degree of staining solution, including zooplankton and phytoplankton.

3. SAMPLE COLLECTION AND TRANSPORTATION

Samples for organisms ≥ 50µm in minimum dimension will be collected in custom constructed zooplankton nets. The nets are built by Institute of Hydrobiology, Chinese Academy of Sciences to our specifications. The mesh size will be 35µm nylon wire material, with all seams double stitched, the diagonal of the mesh size will be 50µm. The description of sample collection is fully elaborated elsewhere.

The zooplankton nets will be rinsed with filtered sample water at the end of each concentrating operation to ensure all organisms are collected in the cod-end. The net contents will be transferred to clean, wide-mouth glass jars to a final volume of nominally 50 mL (measured volumetrically).

4. MATERIALS, INSTRUMENTS AND REAGENTS

- Quantitative liquid pipette
- 4% buffered formalin
- Stereoscopic microscope
- Dissecting needle
- Sedge wick Rafter counting chamber, 1 mL
- Janus Green B solution

5. ANALYTICAL PROCEDURE

After adding Janus Green B solution and formalin, place the sample 24 h without disturbance. We then concentrated the samples to 50mL. Subside the sample for at least 12 hours, and then absorb 1 mL sediment onto a Sedge wick Rafter counting chamber. Numbers of living and dead individuals are counted at 80x magnification using microscopes with bright field (white light) illumination. We judge survival or death of the organisms by observing the color of cells, and

once cell is damaged, the absorbing neutral red may disappear. At last, record the number of living cells with Janus Green B.

After finishing counting all the subsamples, supply 4% buffered formalin to store and carefully identified the species for organisms ≥ 50μm in lab.

6. REFERENCES

Huang Zong-guo. Marine Species and their distribution in China. Beijing: Maritime Press, 2008.
National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.7) - Part 7: Ecological survey for offshore pollution and biological monitoring.

Organisms ≥ 50μm analysis log sheet

The project name			
Test date		Sampling site	
Sample number		Sample State	
Sampling time		The volume of filtered water	
The volume after		The volume of	
Starting time of testing		Ending time of	
Detection conditions	Temperature： ℃，Humidity： %RH		
Instrument	light microscope（SHOU-BWEQ-02）、anatomize lens（SHOU-BWEQ-04）		
Testing standards	GB17378.7-2007, Standard Operating Procedure for Organisms ≥50μm Sample Analysis		
Testing results（number of the living individuals）			
Density of living organisms（ind./m^3）			
Note：Density of living organisms= number of the living individuals / the volume of filtered water			

Analyst: Assessor: Approver:

Appendix B Standard Operating Procedure for Organisms ≥10~50 μm Sample Analysis

1. Scope and Application

This method is suitable for analyzing viable phytoplankton less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension. The sampling, pretreatment of samples, analyzing and quality control is described. The maximum dimension on the smallest axis without thorn, antennae and flagella is ≥10 μm and <50 μm.

Here we describe a method using an algae staining solution to tag viable cells. Estimate the living or dead cell by the staining degree of staining solution. Staining solution can be absorbed by living cells. Once cell is damaged, the absorbing staining solution may disappear. In this way, we can judge the living or dead cells.

2. Sample Collection, Processing and Preservation

2.1 Sample collection

According to G8 guideline of the IMO, 3 × 1 L samples of influent water, 3 × 10 L samples of discharge treated water and control water should be sampled for analyzing organisms 10 - 50 μm.

2.2 Sample processing

The sampled water is slowly sieved through plankton net (10μm diagonal dimensions). The remained organism on the net is washed by filtered water for at least three times. Collect the organisms in a 60 mL bottle. Add 3 drops of staining solution. After 15 min, add 2 mL formalin to the concentrated sample.

2.3 Sample preservation

After subsampled and analyzed, we add formalin to the concentrated sample. Samples with formalin can be preserved for three to six months.

3. Apparatus

Microscope (Leica DM 2500; 100x, 200x, 400x)
Plankton counting chamber (0.1 mL)
Pipette (0.1 mL)

4. Reagents

Neutral red staining solution (should be stored in dark, at -20°C for one year)
Formaldehyde solution (Formalin, 37 - 40%)

5. Analytical Procedure

After adding neutral red and formalin, place the sample 24 h without disturbance. We then concentrated the samples to 50mL. Stained subsamples are shaken up and down, and then absorb 0.1 mL onto a plankton counting chamber with cover glass. Numbers of living and dead

cells are counted at 400x magnification using microscopes with bright field (white light) illumination. We judge survival or death of the organisms by observing the color of cells, and once cell is damaged, the absorbing neutral red may disappear. At last, record the number of living cells with neutral red.

6. References

National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.7) - Part 7: Ecological survey for offshore pollution and biological monitoring.

Organisms ≥ 10μm and <50μm analysis log sheet

The project name		Project number	
Date of entrustment		Test date	
Sample number		Sample State	
Testing standards	GB17378.7-2007；Standard Operation Procedure of testing 10-50μm		
Detection conditions	Temperature： ℃，Humidity： %RH		
Instrument	Fluorescence microscopy、plankton counting chamber		
Sample receiving time		The volume after filtered mL(V_1)	
Sample volume L（V）		The start and end time of testing	

Testing Results					
First	Testing Volume(V2)：	Second	Testing Volume (V2)：	Third	Testing Volume (V2)：
Number of living organisms（cells）X		Number of living organisms（cells）X		Number of living organisms（cells）X	
Density（cells/ml）D_1		Density（cells/ml）D_2		Density（cells/ml）D_3	
Average density（cells/ml）D^1					

Note：Density（D）$=X*V_1/(1000V*V_2)$

Average density（D^1）$=(D_1+D_2+D_3)/3$

Analyst: Assessor: Approver:

Appendix C Standard Operating Procedure for Heterotrophic Bacteria Analysis

1. Method Overview

Plate count method is one of the most simple and effective methods testing the total number of heterotrophic bacteria. The test result of this method is very intuitive, which can get results with macroscopic observation of visible colony on the petri plate. We choose 2216E flat-panel test salt samples, and the nutrient agar plate used in the detection of salt-free samples.

The single bacteria cultivated on plate nutrient medium could form the visible colonies. One colony represents one cell. Relying on counting the number of visible colonies, the plate count method could get the number of bacteria.

The keys to count bacteria are: to separate bacteria on sample to single cell as far as possible; prepare diluents of different concentrations and evenly inoculate certain volume of the diluents on petri plate with solid nutrient medium (hereinafter to be referred as plate).

2. Method References

The National Standard of the Specification for Marine Monitoring—Part 7: Ecological Survey for Offshore Pollution and Biological Monitoring (GB 17378.7-2007/10.1).
Standard Examination Methods for Drinking Water-Microbiological parameters (GB/T 5750.12-2006/1.1).

3. Instruments and Reagents

- Tween-80 solution: mix 1 mL Tween-80 with 2000 mL distilled water.
- 2216E nutrient medium: The components of 2216E nutrient medium: 5 g peptone, 1 g yeast extract, 0.1 g ferric phosphate, 20 g agar; 1000 mL aged seawater.
  Preparation: heat up above substances for dissolution and use NaOH solution to adjust pH value to 7.6. Put the solution into conical flask and place them into autoclave sterilizer for disinfection under the condition of 121°C (about 105 kpa) for 20 min. After that, pour nutrient medium into the disinfected plate and the volume of nutrient medium in every plate is about 15 mL. When the samples cooled and solidified, put them into refrigerator for perseveration.
- Nutrient-Agar-Medium: The components of nutrient agar medium: 10 g peptone, 3 g beef extract, 5 g NaCl , 10 - 20 g agar, 1000 mL aged seawater.
  Preparation: heat up above substances for dissolution and adjust pH value to 7.4 - 7.6. Put the solution into conical flask and place them into autoclave sterilizer for disinfection under the condition of 121°C (about 105 kpa) for 20 min. After that, keep the medium at 50°C until used.
- Constant temperature incubator
- Autoclave sterilizer
- Petri dish: diameter is 9 cm
- Pipet: 1 mL
- Wild-mouth sampling bottle:1000 mL
- Conical flask
- pH meter
- Super clean bench
- Common used instruments and equipment in laboratory

4. Analytical Procedure

- Add 1 mL Tween-80 solution to per 100 mL water sample. Shake well to separate bacteria in sample to single cell.
- 1 mL water sample is used to preparing the 10 times diluted water sample. And then mix the 10 times diluted water sample thoroughly. Following the same procedure, continuous dilution should be done until getting the required extent which depends on the amount of bacteria in water sample. The proper bacterial colony in every plate should in range of 30 - 300 cells. Each dilution extent should have three duplicate plates.
- After inoculating the samples on plates, place the plates upward for at least 20 - 30 min.
- Place the plates into the 25°C constant temperature incubator for 7 days' cultivation. If the samples were fresh water, place the plates into the 37°C constant temperature incubator for 2 days' cultivation.
- Thereafter, take the plate out to count bacterial colony.

5. Data Analysis and Calculations

- Bacterial colony count method
  —If there is large lawn on the plate, the count is invalid. Select the plate which has the total number of colonies between 30 and 300. To obtain results, multiply the average number of colonies per plate by the dilution extent.
  — If the average numbers of colonies in two dilution factors are in the range of 30- 300 colonies per plate, the count is determined by the ratio of two numbers. If the ratio is less than 2, the count takes the average number; if the ratio is greater than 2, the count takes the fewer colony number.
  — If plates from all dilutions have more than 300 colonies, compute the count by the multiplying the average colony number of the least dilution factor plate (the highest concentration plate) by the dilution factor.
  — If plates from all dilutions have less than 30 colonies, compute the count by the multiplying the average colony number of the highest dilution factor plate (the least concentration plate) by the dilution factor.
  — No colonies – if plates from all dilutions of any sample have no colonies and found no contamination, report the count as less than one times the dilution factor. For example, if the minimum dilution factor is 1:100, report the count as less than 100.
- Results will be recorded in the sheet.

6. ATTENTION

The following attentions should be noticed: The bacteriological testing should strictly follow the sterile operation. The collected sample should be tested in time. The allowed storage time is no more than 2 hours. Otherwise, the sample must be stored in ice bottle, but the storage time is not more than 6 hours. Plates should be prepared in advance, or the water on plate would influence the testing results.

Total heterotrophic bacteria detection log sheet

Sample No.	Time		The number of colonies					The density of total heterotrophic bacteria (cfu/mL)
	Sampling time	Detection time	The volume of sub-sample: 0.1 mL					
			0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Remark:								

Analyst: Assessor: Approver:

Appendix D Standard Operating Procedure for *Escherichia coli* Analysis

1. Method Overview

This SOP using multiple tube fermentation method to test a group of aerobic and facultative anaerobic gram-negative bacillus aerobic that can produce gas or acidic after incubation in 36±1°C for 24±2h and subsequently ferment lactose and produce gas within 24 ± 2 hours in EC-MUG broth after incubation at 44.5 ± 0.2°C.The cultivated EC-MUG tube is exposure to the wavelength 366nm ultraviolet (UV) light (6W). The presence of bright blue fluorescence is considered a positive response for *E. coli*.

2. Method References

- The testing method is adopted the multiple-tube fermentation technique in accordance with 'The specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring' (GB17378.7-2007/9.1)
- Standard examination methods for drinking water-Microbiological parameters (GB/T5750.12-2006/2.1, 4.1).

3. Instruments and Reagents

- Medium and reagent
  - Lactose peptone broth
  - Double enrichment lactose peptone culture
  - Eosin-methy blue agar medium
  - Gram stain solution
  - EC-MUG broth
- Instruments
  - Constant temperature incubator:36±1°C; 44.5±0.2°C
  - Refrigerator : 0-4°C
  - Balance
  - Plate: diameter is 9 cm
  - Test tube
  - Graduated pipette:1 mL,10 mL
  - Erlenmeyer flask
  - Small pour tube
  - Glass slide
  - Ultraviolet lamp: 366 nm wavelength, 6W
  - Sterile metal inoculation loop

4. Analytical Procedure

The collected sample should be transported at low temperature to laboratory immediately. Start the examination preferably immediately after sampling. If the samples are kept at ambient temperatures, the examination shall begin within 6 h after taking the sample.

Lactose fermentation test: Shake water sample vigorously 25 times before testing or dilution so as to make sample mixed well. Use the sterile pipet to put 1 mL water sample into the 9 mL sterile dilution solution to form the 10 times dilution water sample. And then shake the diluted sample well. Following the same procedure, make the 100 and 1000 times dilution samples. In the process of above dilution, the sterile pipet needs to be replaced every time. Then inoculate

10 mL sample into 10 mL double lactose peptone medium. Inoculate 1 ml sample into 10 mL single lactose peptone medium. Besides, inoculate 1 ml dilution samples with dilution factors of 10^{-1}, 10^{-2}, 10^{-3} into 10 mL single lactose peptone medium, respectively. Every dilution factor should be inoculated in five tubes. Incubate the inoculated tubes at 36±1°C for 24±2 h.

Isolated culture: If no gas or acidic reaction is evident, report negative results of the total coliform; If gas or acidic reaction is evident, to carry out procedures as follow: Inoculate the fermentation tubes into the plate having eosin methylene blue agar at 36±1°C for 18 to 24 h; Observe colonial morphology and select the required colonies as objects to do the experiments of gram staining, microscopic observation and demonstration. The required characteristics are dark purple black with a metallic surface sheen, purple black without or with little metallic surface sheen and pale purple pink with darker center.

Confirmatory test: If the result of staining microscopic observation shows gram-negative bud less bacillus and gas or acidic reaction is evident after inoculation of lactose peptone medium and incubation at 36±1°C for 24±2 h, it demonstrates the presence of the coliform group.

Inoculation: Test *Escherichia coli* in initial fermentation tubes of above multiple-tube fermentation technique. Use the sterile metal inoculation loop or sterile cotton swab to inoculate the liquid inside fermentation tube into the EC-MUG tube.

Cultivation: Incubate inoculated EC-MUG tube in an incubator at 44.5±0.2 °C for 24±2 h. If EC-MUG tubes are incubated in a water bath, they should be incubated after inoculation in 30 min. Make liquid level the water bath higher than liquid level of EC-MUG tubes

Observation of result: The cultivated EC-MUG tube is exposure to the wavelength 366nm ultraviolet (UV) light (6W). The presence of bright blue fluorescence is considered a positive response for *E. coli*.

5. Data Analysis and Calculations

The presence of bright blue fluorescence is considered a positive response for *E. coli*. Calculate the number of positive EC-MUG tubes and look up the most possible number (MPN) table (Supplement A) to get the MPN of *E. coli*. Report the result as MPN/100 mL.

Results of diluted sample after looked up table should be multiplied by the dilution ratio. If all of EC-MUG tubes are negative, then the total coliform bacteria do not checked out. The results are filled in the sheet.

Supplement A

MPN retrieval table of Total coliforms
Total inoculation amount: 55.5 mL, five copies: 10 mL water sample, five copies: 1 mL water sample, five copies: 0.1 mL water sample

Inoculation amount (mL)			Total coliform (MPN/100 mL)	Inoculation amount (mL)			Total coliform (MPN/100 mL)
10	1	0.1		10	1	0.1	
0	0	0	＜2	1	0	0	2
0	0	1	2	1	0	1	4
0	0	2	4	1	0	2	6
0	0	3	5	1	0	3	8
0	0	4	7	1	0	4	10
0	0	5	9	1	0	5	12
0	1	0	2	1	1	0	4
0	1	1	4	1	1	1	6
0	1	2	6	1	1	2	8
0	1	3	7	1	1	3	10
0	1	4	9	1	1	4	12
0	1	5	11	1	1	5	10
0	2	0	4	1	2	0	6
0	2	1	6	1	2	1	8
0	2	2	7	1	2	2	10
0	2	3	9	1	2	3	12
0	2	4	11	1	2	4	15
0	2	5	13	1	2	5	17
0	3	0	6	1	3	0	8
0	3	1	7	1	3	1	10
0	3	2	9	1	3	2	12
0	3	3	11	1	3	3	15
0	3	4	13	1	3	4	17
0	3	5	15	1	3	5	19
0	4	0	8	1	4	0	11
0	4	1	9	1	4	1	13
0	4	2	11	1	4	2	15
0	4	3	13	1	4	3	17
0	4	4	15	1	4	4	19
0	4	5	17	1	4	5	22
0	5	0	9	1	5	0	13
0	5	1	11	1	5	1	15
0	5	2	13	1	5	2	17
0	5	3	15	1	5	3	19
0	5	4	17	1	5	4	22
0	5	5	19	1	5	5	24

2	0	0	5	3	0	0	8
2	0	1	7	3	0	1	11
2	0	2	9	3	0	2	13
2	0	3	12	3	0	3	16
2	0	4	14	3	0	4	20
2	0	5	16	3	0	5	23
2	1	0	7	3	1	0	11
2	1	1	9	3	1	1	14
2	1	2	12	3	1	2	17
2	1	3	14	3	1	3	20
2	1	4	17	3	1	4	23
2	1	5	19	3	1	5	27
2	2	0	9	3	2	0	14
2	2	1	12	3	2	1	17
2	2	2	14	3	2	2	20
2	2	3	17	3	2	3	24
2	2	4	19	3	2	4	27
2	2	5	22	3	2	5	31
2	3	0	12	3	3	0	17
2	3	1	14	3	3	1	21
2	3	2	17	3	3	2	24
2	3	3	20	3	3	3	28
2	3	4	22	3	3	4	32
2	3	5	25	3	3	5	36
2	4	0	15	3	4	0	21
2	4	1	17	3	4	1	24
2	4	2	20	3	4	2	28
2	4	3	23	3	4	3	32
2	4	4	25	3	4	4	36
2	4	5	28	3	4	5	40
2	5	0	17	3	5	0	25
2	5	1	20	3	5	1	29
2	5	2	23	3	5	2	32
2	5	3	26	3	5	3	37
2	5	4	29	3	5	4	41
2	5	5	32	3	5	5	45
4	0	0	13	5	0	0	23
4	0	1	17	5	0	1	31
4	0	2	21	5	0	2	43
4	0	3	25	5	0	3	58
4	0	4	30	5	0	4	76
4	0	5	36	5	0	5	95
4	1	0	17	5	1	0	33

4	1	1	21	5	1	1	46
4	1	2	26	5	1	2	63
4	1	3	31	5	1	3	84
4	1	4	36	5	1	4	110
4	1	5	42	5	1	5	130
4	2	0	22	5	2	0	49
4	2	1	26	5	2	1	70
4	2	2	32	5	2	2	94
4	2	3	38	5	2	3	120
4	2	4	44	5	2	4	150
4	2	5	50	5	2	5	180
4	3	0	27	5	3	0	79
4	3	1	33	5	3	1	110
4	3	2	39	5	3	2	140
4	3	3	45	5	3	3	180
4	3	4	52	5	3	4	210
4	3	5	59	5	3	5	250
4	4	0	34	5	4	0	130
4	4	1	40	5	4	1	170
4	4	2	47	5	4	2	220
4	4	3	54	5	4	3	280
4	4	4	62	5	4	4	350
4	4	5	69	5	4	5	430
4	5	0	41	5	5	0	240
4	5	1	48	5	5	1	350
4	5	2	56	5	5	2	540
4	5	3	64	5	5	3	920
4	5	4	72	5	5	4	1600
4	5	5	81	5	5	5	＞1600

Total coliform detection log sheet

Sample No.	Sampling time	Detection time	The number of positive EC-MUG tubes						Total coliform (MPN/100 mL)
			10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Remarks:									

Analyst: Assessor: Approver:

Appendix E Standard Operating Procedure for Intestinal *Enterococci* Analysis

1. Method Overview

Membrane filter method is used to verify and count the enterococcus in the water. A amount of water were filtrated by using the 0.45 μm membrane filter which can prevent the bacteria. The filter is placed on a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and 2, 3, 5- triphenyltetrazolium chloride, a colourless dye, that is reduced to red formazan by intestinal enterococci.

2. Method References

Water quality: Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method (ISO 7899.2-2000).

3. Instruments and Reagents

- Distilled water
- Slanetz and Bartley medium
- Bile-aesculin-azide agar
- Except for disposable glassware
- Sterile membrane filters, with a nominal pore size of 0.45 μm.
- Vacuum pump
- Incubator (capable of being maintained at 36 ± 2°C; 44 ± 0.5°C and 121 ± 3°C)
- Sterile forceps

4. Analytical Procedure

- Preparation of the sample: Start the examination preferably immediately after taking the samples. If the samples are kept at ambient temperatures, the examination shall begin within 6 h after taking the sample. Under exceptional circumstances, it is permissible for the samples to be kept at 5 ± 3°C for up to 24 h prior to examination.

- Filtration and incubation: Filter 100 mL water being examined. The collected sample should be transported at low temperature to laboratory immediately. Shake water sample vigorously 25 times before testing so as to make sample mixed well. Connect the sterile filter device to filter flask. Use the sterile tweezers to put filter membrane on the bottom of filter. Pipette some water sample into filter and open vacuum pump to start extraction filtration. After all the water passes through filter membrane, use 20 - 30 mL of sterile normal saline to wish the edge of filter at least two times, and then turn off the vacuum pump. Open the filter and use sterile tweezers to move the filter membrane to the surface of Slanetz and Bartley medium. Prevent bubbles production between filter membrane and medium. Incubate the plates at 36 ± 2°C for 44 ± 4 h.

- Confirmation and enumeration: After incubation, consider all raised colonies which show a red, maroon or pink color, either in the center or throughout the colony, as typical. If there are typical colonies, transfer the membrane and the colonies, with sterile forceps without inverting it, onto a plate of bile-aesculin-azide agar which has been preheated to 44°C. Incubate at 44 ± 0.5°C for 2 h.

5. Data Analysis and Calculations

- Read the plate immediately after incubate at 44 ± 0.5°C for 2 h. The presence of brownish black around colonies is considered a positive response for enterococcus. Count the identified enterococcus and report the result as cfu/100 mL.
- Record cfu/100 mL scores in intestinal *enterococci* detection and enumeration log sheet.

Intestinal *enterococci* detection and enumeration log sheet

Sample No.	Time		The volume of filtered water (mL)	The number of colony forming unit after 44 h cultured at 36°C	The number of colony forming unit after 2 h cultured at 44°C
	Sampling time	Detection time			
Remarks:					

Analyst: Assessor: Approver:

Appendix F Standard Operating Procedure for *Vibrio Chloerae* Analysis

1. Scope and Application

This method is utilized to detect *Vibrio Cholera* of serogroups O1 and O139 in ballast water.

2. Method Overview

Water sample be inoculated in basic peptone water medium. Place the enrichment medium with samples into incubator for enlargement cultivation at 37°C for 6-8 h. Then the strong and weak nutrient mediums were inoculated, they were used to isolated culture and made cultivated substance morphology observation. Vibrio cholera in different isolation mediums presents different characteristics. Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

3. Sample Collection and Transportation

For isolation and detection of a higher probability *V. cholerae* from the samples, we choose concentrated water samples included plankton samples and water samples.

Water collection bottles should be cleaned with detergent, however the latter must not leave residue, should not be anti-bacterial and they should be pre-sterilized in an autoclave for 15 to 20 minutes at 121°C prior to use. Polypropylene bottles should be used for water samples. A sufficient volume of water and plankton should be collected to insure that appropriate analyses can be performed.

Processing of samples should begin soon after collection (typically within 24 hours of collection). If processing is delayed, the sample should be stored in a cool box at a temperature of 10 to 15°C until processing begins (not to exceed 8 hr). Based on type of examination, samples may require treatments; such as addition of direct viable count (DVC) reagents, before proceeding with further examination and testing. It is recommended that basic physiochemical parameters, e.g., temperature, salinity, pH, dissolved oxygen and conductivity of the water be measured on site at the time of collection as it is known that *V. cholerae* densities can be influenced by such parameters. These parameters can be measured on site.

4. Materials, Instruments and Reagents

- Non-selective enrichment medium -Basic peptone water medium
- No. 4 agar
- Alkaline nutrition agar
- N,N-Dimethyl-p-phenylenediamine dihydrochloride or 1% N,N-Dimethyl-p-phenylenediamine dihydrochloride
- Vibrio cholera O1 in serum
- Vibrio cholera O139 in serum
- Filter paper

5. Analytical Procedure

- The water sample shall be immediately vaccinated in enrichment medium after sampling. In general, we absorb 50 mL water sample into 450 mL enrichment medium. Then place the medium into incubator for enlargement cultivation at 37°C for 6 - 8 h. Put one sterile

inoculation loop under the biofilm of enrichment medium. Streak on a strong nutrient medium (No. 4 agar) and a weak nutrient medium (alkaline nutrition agar), respectively, and then incubate the two plates at 37°C for 18 - 24 h. Vibrio cholera in selective medium presents different characteristics.

- In the strong and weak isolation medium with incubation of 37°C for 18-24 h, the characteristics of Vibrio cholera colony is as follows:

- Colony in the Alkaline nutrition agar (AN): colorless, roundness, transparent or semitransparent, smooth surface, wet, flat or slight convex, neat edge and the diameter of colony is ca. 2 mm in general.
- Colony in the No. 4 agar (No. 4): the characteristics is similar with the colony in alkaline nutrition agar, but most of them show semitransparent; because these nutrient mediums contain tellurite, the center of colonies generally present grey or grey to black, and moreover the color become darker with the increase of cultivation time.
- Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

- Slide agglutination test (ST): Pick suspected colonies from the isolation medium and pure cultivation of suspected colonies was conducted in the non-selective medium at 37°C for 18h~24h. Pure culture was slide agglutination tested with vibrio cholera O1 in serum and vibrio cholera O139 in serum, strain screening will be carried out. If the obvious visible aggregations appear in 1min, and there are no agglutinations in physiological saline, so the judgment is agglutination positive. On the contrary, the judgment is agglutination negative. Each sample should be selected at least more than five suspected colonies that be identified.

- Oxidase test (OT): Fresh cultures of growth on the isolation medium are daubed on a clean filter paper, and then add N,N-Dimethyl-p-phenylenediamine dihydrochloride or 1% N,N-Dimethyl-p-phenylenediamine dihydrochloride. If cultures become pink-purple-blue purple in 1-2 min, and some are purple at last. The judgment is oxidase test positive. If cultures didn't color, the judgment is oxidase test negative.

- The experimental results are filled in the following sheet.

NOTE: If finding positive strains in slide agglutination test and oxidase test, all the positive and suspicious strains should be transported to Shanghai Disease Control Centre immediately to do further detection.

6. Method References

Diagnostic criteria of *Vibrio cholerae* (WS289-2008).

Vibrio cholera **determination log sheet**

Date: Operator: Approved by:

Sample No.	Time		The volume of water sample (mL)	Isolated culture (color reaction)		Positive or negative in Strain identification	
	Sampling time	Detection time		AN	No.4	OT	ST

Alkaline nutrition agar (AN): colorless, roundness, transparent or semitransparent, smooth surface, wet, flat or slight convex, neat edge and the diameter of colony is 2 mm in general.
No. 4 agar (No. 4): the characteristics are similar with the colony in alkaline nutrition agar, but they have badly transparent and most of them show semitransparent; the center of colonies generally presents grey or grey to black, and moreover the color become darker with the increase of cultivation time.
ST— Slide agglutination test (agglutination positive or agglutination negative)
OT—Oxidase test(positive or negative)

Remarks:

Analyst: Assessor: Approver:

Appendix G Standard Operating Procedure for pH Analysis

The testing method use Switzerland METTLER Desktop pH meter to determine pH value of water sample in accordance with Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/26).

1. Scope and Application

This method is applicable to determine pH value of seawater and offshore seawater. Determination should be completed within 6 hours after sampling. If one drop of $HgCl_2$ solution is added into the water sample with the bottle stopper sealed, sample can be preserved for 2 days. Factors such as water chromaticity, turbidity, colloidal particles, free chlorine, oxidants, reducing agents and high salinity have small interference on pH value determination. When pH is higher than 9.5, the considerable Na^+ will cause a big error and make the reading value lower.

2. Principle of the Method

Insert glass-calomel electrode into the water sample to form the battery. Then, pH of the water sample has a linear relationship with and EMF (E) of the battery.

$$pH_s = \mathrm{A} + \frac{E_x}{2.3026RT/F}$$

When glass-calomel electrode is inserted into the standard buffer solution, then A is calculated as follows:

$$\mathrm{A} = \mathrm{pH_s} - \frac{\mathrm{E_x}}{2.3026\mathrm{RT/F}}$$

At the same temperature, measure EMF of the same electrode both in standard buffer solution and the water sample. The pH value of the water sample is calculated using the following equation:

$$pH_x = pH_s + \frac{E_x \times E_s}{2.3026RT/F}$$

Where: *pHx*——pH value of the water sample;
pHs——pH value of the standard buffer solution;
Ex——EMF of glass-calomel electrode in the water sample;
Es——EMF of glass-calomel electrode in the standard buffer solution;
R——gas constant;
F——Faraday constant;
T——absolute temperature K.

3. Reagents

3.1 Standard buffer solution (preparing by using pH standard buffer substance)

3.1.1 Potassium hydrogen phthalate standard buffer solution: c ($KHC_8H_4O_4$) = 0.05 mol/L (pH_s = 4.003 at 25°C);

3.1.2 Mixed standard buffer solution (pH_s = 6.864 at 25°C): 0.025 mol/L potassium dihydrogen phosphate (KH_2PO_4) and 0.025 mol/L disodium hydrogen phosphate (Na_2HPO_4);

3.1.3 Mixed standard buffer solution (pH_s = 7.413 at 25°C): 0.008695 mol/L potassium dihydrogen phosphate (KH_2PO_4) and 0.03043 mol/L disodium hydrogen phosphate (Na_2HPO_4);

3.1.4 Borax solution standard buffer solution (pH_s = 9.182 at 25°C): c ($Na_2B_4O_7 \cdot 10H_2O$)= 0.010mol/L;

3.2 Saturated potassium chloride solution: Weigh 40 g potassium chloride (KCl). Mix with 100 mL water thoroughly. Then, store the solution in reagent bottle. (The solution should be co-existed with solid KCl).

3.3 Mercuric chloride solution (25 g/L): Weigh 2.5 g mercuric chloride ($HgCl_2$). Dissolve it into water and dilute to 100ml. Mix thoroughly. Then, store it in brown reagent bottle.

4. Instruments and Equipment

pH meter: Accuracy of 0.01, with a glass electrode and calomel electrode
Polyethylene wash bottle: 500 mL
Thermometer: (0-60) °C
Beaker: 150 mL
Brown drop bottle: 60 mL
HDPE bottle: 100 mL, 500 mL, 1000 mL
Wide-mouth bottle: 50 m
Brown reagent bottle: 250 mL, 50 mL, 1000 mL;
Volumetric flask: 250 mL, 50 mL, 1000 mL
Common used instruments and equipment in laboratory.

5. Procedure

Start up and preheat the instrument for 20 minutes. Shift pH-mV selector switch to “pH”. Place the beaker holder, electrode clip, etc. Fix the glass electrode and calomel electrode on the clip (the bottom of the calomel electrode should be slightly lower than that of the glass electrode). Insert and connect the electrode to the corresponding jacks and terminals. Wash electrode with water (there should be potassium chloride crystals in calomel electrode and remove the rubber plug on the bottom of the electrode). Dry it with filter paper, and then insert the electrode to the calibrated standard buffer solution. Before testing, use standard buffer solution for calibration. The standard buffer solution which has the similar pH value with that of the test solution would be selected as the calibration solution. If the pH range of the test solution is unknown, choose the phosphate standard buffer solution. The calibration procedure is as follows:
(1) Keep the scale of “temperature compensator” of the instrument the same with the temperature of the solution.
(2) Zero setting, to make the instrument reading between ± 0.
(3) Press the “Reading” switch and adjust the “Calibration” switch to read the pH value at present temperature. Note that electrode potential must be kept fully balanced and stable when calibration.
(4) After calibration, release the “Reading” switch. The “Calibration” switch should not be rotated casually any more, otherwise the instrument should be re-calibrated.

When sample analysis, take out electrode and wash the bottom of the electrode with distilled

water. After that, dry it with filter paper and insert the electrode to the test solution. Rotate the beaker containing the solution from time to time to keep the electrode potential fully balanced. Keep the scale of "temperature compensator" of the instrument the same with the temperature of the solution. Zero setting. Press the "Reading" switch and read pH value of sample. Then release the "Reading" switch and write down the data on the datasheet. The instrument should be re-calibrated if it is used for 2-3 hours or the temperature changes more than 2°C. After determination, take out the electrode and wash it with distilled water until it is clean. Cap the rubber plug of calomel electrode and store it in electrode box. Glass electrode is soaked in distilled water.

6. Computing and Reporting

Depending on the requirements of datasheet, fill in the datasheet and complete the data calculation. Convert the pH value measured in laboratory to that of onsite sample. Calibrate temperature and pressure according to the following formula:

$$pH_w = pH_m + \alpha\,(t_m - t_w) - \beta \times d$$

Where: pH_w, pH_m = pH value on the scene and in the laboratory respectively;
t_w, t_m = water temperature on the scene and in the laboratory respectively, °C;
d = the depth of water sample, m;
α = temperature correction factor;
β = pressure correction factor;
$\alpha(t_m - t_w)$ value can be found in temperature correction table and pressurecorrection coefficient method.

7. Attentions

Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is de-ionized water or equivalent pure water. The "reading" switch, glass electrode jack, calomel electrode, terminal, etc. should be kept dry and clean.

Common faults and solutions:
a) If the deterioration of buffer solution leads to pH value change (floccule appears, etc.), the buffer solution should be replaced. To prevent deterioration of the buffer solution, it is better to add a few small thymol crystals to the solution beforehand;
b) If the passivation of glass electrode results in poor response of pH value. The pH meter can be soaked in 6 mol/L hydrochloric acid or 20% ammonium hydrogen fluoride (NH_4HF_2). If no improvement still, replace the glass electrode.
c) If the bubbles in potassium chloride solution inside calomel electrode lead to open circuit or test instability, the bubbles in the liquid column should be removed and fill the liquid column with saturated potassium chloride solution.
d) If electrode wiring doesn't connect well or the instrument is affected with damp which leading to meter hand is unstable, the wiring should be reconnected or dry the instrument. The electrode must be immersed in the solution during measurement, otherwise it is likely to cause open circuit and break the instrument; before measuring of each batch of water samples, the instrument must be calibrated with phosphate buffer solution (refer to 3.1.2). If the deviation between measured value and reference value is more than ±0.01, the instrument should be re-calibrated; the bulb of glass electrode is very thin, so not to collide with hard objects to make it broken. Calomel electrode should be slightly lower than the glass electrode when in use; new glass electrode should be soaked in water for 1-2 days before use; after determination of turbid water, the electrode should be washed immediately.

pH analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH		
Instrument	pH meter, thermometer		
Instrument model	FE20		
Detection date and time			
pH value of control:		pH value of control (detection):	

Test Results

Sample ID	Water temp. (t_m, °C)	Detection value (pH_m)				Water temp. in situ (t_w, °C)	Correction		pH_w in situ
		1	2	3	Mean		t_m-t_w	$\alpha(t_m-t_w)$	
Calculation	$pH_w = pH_m+\alpha(t_m-t_w)$ (depth of water sample is within 500 m)								
Remarks:									

Appendix H Standard Operating Procedure for Turbidity Analysis

The testing method use Shanghai Xinrui Photoelectric Turbidimeter to determine turbidity of water sample in accordance with the Specification for Marine Monitoring-Part 4: Seawater Analysis (GB17378.4-2007/30.1).

1. Scope and Application

This method is applicable to determine turbidity of seawater in ocean and offshore area. The turbidity of 1 L pure water containing 1 mg Kaolin is defined as 1° in this method. Both the rapid sinking debris and thick sediment in water sample can be determined as turbidity.

2. Principle of the Method

Turbidity can be determined by comparing the transmitted light intensity of the water sample with that of the turbidity-free water, when certain light beam irradiate water sample.

3. Reagent

3.1 Turbidity-free water: Filtrate distilled water or deionized water through 0.2 μm membrane and store the water in polyethylene bucket. Wash the polyethylene bucket by the filtered water twice, and then abandon the 200 mL initial filtered water. Prepare the turbidity-free water on the sampling day.

3.2 Mercury dichloride ($HgCl_2$) solution (50 g/L): weigh 5.0 g mercury dichloride ($HgCl_2$). Dissolve it into little water and dilute to 100 mL. Store it in brown reagent bottle.

4. Instruments and Equipment

Photoelectric turbidimeter
Reagent bottle with rubber stopper
Common instruments and equipment in general laboratory.

5. Procedure

Start up and preheat the turbidimeter for 15-30 minutes. Use long test-cell to determine the water sample having low turbidity (0-30°). The procedure is as follows:
(1) Zero setting: Pour the turbidity-free water into the cell and face the numbered side of the cell with the right side of the tank. Close the cap. Rotate the vernier adjustment knob slowly and adjust the hand to zero on the right side of dial. Take out the turbidity-free water from the cell.
(2) Water sample determination: Pour the sample water into the cell to the standard line. Put the cell back to the tank and face the numbered side of the cell with the right side of the tank. Close the cap. Record the reading directly.

Use short cell to determine the water sample having high turbidity (20-100°). The procedure is as follows:
(1) Pour the turbidity-free water into the high turbidity measuring cell to the standard line. Insert the 20° base plate to the numbered side of the cell and put the cell into the tank with numbered side of the cell facing one side of the tank.
(2) Close the cap of the tank. Rotate the vernier adjustment knob slowly and adjust the hand to 20° scale value on the right side of dial.

(3) Take out the 20° base plate and pour the sample water into the cell to the standard line. Put the cell into the tank and close the cap. Record the reading directly.
(4) When the turbidity of water sample exceed l00°, it can be diluted by turbidity-free water before determination.

6. Computing and Reporting

Record the data in datasheet. If the water sample is diluted, calculate its concentration according to the following formula:

$$T_u = \frac{F \times (A + C)}{C}$$

Where: T_u = Turbidity unit of the water sample, °;
F = Turbidity unit calculated according to working curve, °;
A = Volume of turbidity-free water, mL;
C = Volume of original water sample, mL.

7. Attentions

- Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is turbidity-free water or equivalent pure water;
- Turbidity determination should be quick. The whole period from pouring the mixed water sample or standard sample to the cell to recording the reading should be within 3 minutes;
- The water sample should be determined on the sampling day. If the sample needs to be kept for longer time, it should be stored in dark place for 24 hours. If 0.5 g/L $HgCl_2$ fixative is added into the sample, it can be kept for 22 days.

Turbidity analysis log sheet

Date: Operator: Approved by:

Project name		Project No.	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH		
Instrument	Turbimeter		
Instrument model		Detection date and time	
Test Results			
Sample ID	Absorbance (A_w) or turbidity (°)		
	1	2	Mean
Remarks:			

Appendix I Standard Operating Procedure for TSS Analysis

1. Method Overview

When the certain amount of water pass through 0.45 μm filter membrane, weigh the suspended matter which left on the filter membrane to calculate the concentration of suspended matter in water.

2. Method References

The specification for marine monitoring – Part 4: Seawater analysis (GB 17378.4-2007).

3. Analytical Procedures

3.1 Filter membranes are baked at 40-50°C and keep them at constant temperature for 6-8 h. After that, take them into the silica gel drier for 6-8 h.

3.2 Count the number of blank calibrated membranes and point color dot on membrane to distinguish the filter membranes of water samples.

3.3 Weigh filter membranes and put them into the numbered filter membrane boxes.

3.4 Use stainless steel tweezers to put the weighed water sample filter membrane (W_2) on the weighed blank calibrated membranes (W_b), and place both of them into filter.

3.5 Mix water sample thoroughly. Use measuring cylinder to take a certain volume of water sample (If the concentration of suspended matter is greater than 1000 mg/L, take 50-100 mL of water sample; if the concentration of suspended matter is less than 100 mg/L, take 1-5 L of water sample).

3.6 Turn on vacuum pump. Pour water sample into the filter. Use distilled water to wash the measuring cylinder and the washing water is also poured into the filter. In order to wash away salinity, after pumping water out at the first time, use distilled water to wash filter membrane for three times (50 mL per each time), and then pump water out again.

3.7 Use stainless steel tweezers to take the filter membranes back to their original filter membrane boxes. Either place the boxes into oven and dry them at 50°C, or dry them by air drying in natural environment. Cap the filter membrane box and preserve them in order. Bring these boxes to laboratory.

3.8 Drying: place filter membranes into the electro-thermal constant temperature dry box (40-50°C) to dewater filter membranes at constant temperature for 6-8 h. Thereafter, take filter membranes out and place them into the silica gel drier. After 6-8 h, weigh them (W_1).

3.9 Weighing: select the sense of analytical balance on basis of the quality of suspended matter. If the quality is less than 50 mg, select the one hundred thousandth balance; if the quality is greater than 50 mg, select the one ten thousandth balance. Weighing should be quickly. Two times of weigh, before and after filtration, should keep the similar conditions in terms of temperature and humidity in balance room.

4. Blank calibration of filter membrane

During filtration, the glass fiber filter membrane might be dissolved leading to weight loss. Therefore, it must carefully deal with the weighing work of blank calibration sample.

5. Data Analysis and Calculations

5.1 The concentration of suspended matter should be calibrated by the following formula:

$$\rho = \frac{W_1 - W_2 - \Delta W}{V}$$

where: ρ = the concentration of suspended matter, (mg/L); W_1 = the total weight of suspended matter, water sample and filter membrane, (mg); W_2 = the weight of water sample and filter membrane, (mg); W = the calibration value of blank calibration sample, (mg); V = the volume of water sample, (L).

The calibration value of blank calibration sample can be calculated by the following formula:

$$\Delta W = \frac{1}{n} \times \sum^{n} (W_n - W_b)$$

where: W_n= the weight of the blank calibration filter membrane after filtration, (mg); W_b= the weight of the blank calibration filter membrane before filtration, (mg); n = the number of the blank calibration filter membrane; ΔW should be negative number.

Total suspended solids (TSS) analysis log sheet

Date: Operator: Approved by:

Sample No.	Time		Filter weight (mg)		V (L)	TSS (mg/L)
	Sampling time	Detection time	Before filtration (W_1)	After filtration (W_2)		

Calculation formula:

$$\rho = \frac{W_2 - W_1}{V}$$

ρ = Total suspended solids concentration, (mg/L);
W_1 = Membrane weight, (mg);
W_2 = Membrane weight, after filtered, (mg);
V = The volume of filtered sample, (L).

Appendix J Standard Operating Procedure for POC Analysis

1. Method Overview

Spectrophotometry measures the decreased absorbance of dichromate solution to determine the amount of oxidizable carbon. The reaction is wet oxidation between acidic dichromate and carbon. The measurement range (measured as C) is 0.01-4 mg/L.

2. Method References

Spectrophotometry is adopted as the testing method in accordance with Gulf ecosystem observation methods (China Environmental Science Press, 2005/4.5.14.1)

3. Instruments and Reagents

3.1 Spectrophotometer

3.2 Filter membrane: 0.45 μm of pore size, GF/C glass fiber filter membrane (diameter is 47 mm) and standard micropore filter equipment. Filter membrane should be packed inside aluminum foil and placed into muffle furnace for 24 h heating at 450-500°C to remove oxidizable substances (the temperature should be not more than 500°C; otherwise the filtration ability would be changed).

3.3 Sulfuric acid/dichromate oxidant: dissolve 4.84 g potassium dichromate ($K_2Cr_2O_7$) in 20 mL distilled water, and put this solution slowly into 1000 mL volumetric flask which has 500 mL concentrated sulfuric acid (AR). After the mixed solution cools down, fix volume of 1000 mL volumetric flask by adding concentrated sulfuric acid, and then preserve this solution in glass bottle with stopper. This solution is stable.

3.4 Mixing cylinder with stopper: 50 mL

3.5 Volumetric flask: 1000 mL

3.6 Phosphoric acid (H_3PO_4, AR): 70%.

3.7 Sodium sulfate solution: dissolve 45 g sodium sulfate (Na_2SO_4) in 1000 mL distilled water.

4. Analytical Procedures

4.1 Put the pretreated glass fiber filter membrane in the standard micropore filter equipment. Connect filter equipment with vacuum pump that can control the vacuum degree. Pipette some seawater sample (0.5-2 L in general) to filter equipment. After filtration, the air suction of filter membrane lasts for 1 min. Add 2 mL sodium sulfate solution and then repeat air suction. Add 2 mL sodium sulfate solution again and take out filter membrane during air suction.

4.2 Put the filter membrane into a 30-mL beaker. Add 1.0 mL phosphoric acid and 1.0 mL distilled water into the beaker. Mix thoroughly. Put the beaker on heating plate for heating 30 min at 100-110°C. Beaker should be covered using glass dish when heating.

4.3 Add some sulfuric acid/dichromate oxidant and distilled water. The addition volume of oxidant can be determined by the expected carbon quantity as shown in the following table:

Evaluation of the dosage of oxidation

Expected carbon quantity (μg)	Oxidant (mL)	Distilled Water (mL)	Final Volume (mL)	Length of Cuvette (cm)
<300	2.00	0.8	100	10
300-700	4.00	1.6	50	2.5
700-2000	10.00	4.0	50	1

4.4 Mix thoroughly. Every beaker should be covered by glass dish and be heated at 100-110°C for 60 min.

4.5 Place cool, and move the solution and glass fiber filter membrane to the proper measuring cylinder. Distilled water is used to wash beaker wall and pour the wash water into the measuring cylinder. Plug the bottle tightly and mix upside down thoroughly. Waiting for the solution to cool down at room temperature and the filter membrane would sink to the bottom of the measuring cylinder.

4.6 At the wavelength of 440 nm, measure the decreased absorbance of blank solution using practical water sample as the reference solution

4.7 Because Cr III absorbs the decreased light, the measured absorbance should be calibrated by the following formula:

$$\mathrm{E} = 1.1 \times E_f$$

where: E_f = the difference of decreased absorbance between practical water sample and blank solution in section 4.6.

4.8 POC (measured as C) could be computed by the following formula, μg/L:

$$POC = \frac{E * F * v}{V}$$

where: V = the volume of the filter sample, L; v = the required volume of oxidant in section 4.3; F = 275 (section 6.3).

5. Blank Test

Every unknown sample should carry out the blank determination using the filter membrane and oxidant. Using distilled water as reference solution, the decreased absorbance of blank should be in the range of 1-1.1.

6. Calibration

6.1 Standard solution of glucose: dissolve 7.50 g glucose and some quantities of mercuric chloride crystal ($HgCl_2$) to form 100 mL solution. The stability of the solution could be preserved in freezer for several months. If the solution turns up turbidity, it cannot continue to be used.

6.2 Add distilled water to dilute the solution from 10 mL to 1 L so that 1.00 mL solution has 300 μg C.

6.3 Put one piece of glass fiber filter membrane and 1 mL phosphoric acid into a 30 mL beaker.

Heat them at 100-110°C for 30 min. Add 10 mL oxidation and 4 mL dilute glucose solution into every beaker. Following the steps in sections 4.4-4.7, factor F could be computed as follows:

$$F = \frac{120}{E_3}$$

where: E_3 = the average decreased absorbance of the calibrated Cr III at 440 nm wavelength. The computed result of F is about 274.

Particulate organic carbon (POC) analysis log sheet

Date: Operator: Approved by:

Sample No.	Time		E_f	V	ν	F	POC (mg/L)
	Sampling time	Detection time					

Calculation formula:

$$POC = \frac{E * F * v}{V}$$

E = 1.1* E_f
F = 274
V = the volume of the filter sample (L)
ν = the required volume of oxidant (mL)

Appendix K Standard Operating Procedure for DOC Analysis

1. Method Overview

The determination of dissolved organic carbon adopts the total organic carbon instrument method. This method is applicable to measure total organic carbon (TOC) and dissolved organic carbon (DOC) in seawater.

2. Method References

The Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007).

3. Instruments and Reagents

- Potassium peroxydisulfate ($K_2S_2O_8$).
- Phosphoric acid (H_3PO_4)
- Sodium carbonate (Na_2CO_3).
- Sodium bicarbonate ($NaHCO_3$)
- Hydrochloric acide (HCl)
- Potassium hydrogen phthalate
- Anhydrous sodium sulfate
- Carbon-free water: distilled water is imported into the glass reflux equipment, and 10 g potassium sulphate (K_2SO_4) and 2 mL phosphoric acid are added to 1 L water. Besides, add some zeolite. After heating and refluxing for 4 h, use ground glass receiving device to collect the fractionated water. When the water cools down to the room temperature, use it immediately.
- Sodium carbonate: prebake sodium carbonate at 500°C for 30 min, and then put it into the desiccator which has anhydrous sodium sulfate inside. Store it for later use.
- Hydrochloric acid solution: 2 mol/L
- Phosphoric acid solution (25%): use water to dilute 25 mL phosphoric acid solution to 100 ml
- Standard stock solution of total carbon (1000 mg/L): dissolve 2.1250 g phthalic acid which is prebaked at 115°C for 2 h into water. Move the solution into 1000 mL volumetric flask and fix the volume by adding water. Mix thoroughly.
- Standard stock solution of inorganic carbon (1000 mg/L): dissolve 4.4100 g sodium carbonate and 3.5000 g sodium bicarbonate into water. Move the solution into 1000 mL volumetric flask and fix the volume by adding water. Mix thoroughly
- Standard solution of total carbon: pipette 0.00, 5.00, 10.00 and 15.00 mL of stock solution of total carbon into four 250 mL volumetric flasks, respectively. Fix the volume by adding the no-carbon water. The corresponding concentration of standard solution is 0.00, 20.00, 40.00 and 60.00 mg/L, respectively. Prepare this solution just when using.
- Standard solution of inorganic carbon: pipette 0.00, 5.00, 10.00 and 15.00 mL of stock solution of inorganic carbon into four 250 mL volumetric flasks, respectively. Fix the volume by adding the no-carbon water. The corresponding concentration of standard solution is 0.00, 20.00, 40.00 and 60.00 mg/L, respectively. Prepare this solution right before using.

4. Analytical Procedures

- Calibration curve: Under optimal technical parameters of instrument, inject 0, 20.00, 40.00 and 60.00 mg/L of the standard solutions of total carbon (TC). The instrument would draw the working curve of TC. Similarly, input 0, 20.00, 40.00 and 60.00 mg/L of

the standard solutions of inorganic carbon (IC). The instrument would draw the working curve of IC. In general, making parallel determination for 2–3 times is necessary. Relying on the zero point transfer function of the instrument, move calibration curve to the origin point and save the results.

- Wait for stabilization of instrument and start testing. First test TC value of sample and later test IC value of sample.
- The collected sample should be filtered and analyzed immediately by the preheat Whatman GF/C glass fiber filter membrane. Filtered seawater was analyzed by the instrument. First test TC value of sample and later test IC value of sample. The DOC value is determined by the following formula: DOC = TC – IC.
- When carbon content is 3.00 mg/L, the relative standard deviation of repeatability is 2%; the relative standard deviation of reproducibility is 2%; relative error is 1%.

Dissolved Organic Carbon (DOC) analysis LOG sheet

Date: Operator: Approved by:

Sample No.	Time		TC (mg/L)	IC (mg/L)	DOC (mg/L)
	Sampling time	Detection time			

Remarks:

Appendix L Standard Operating Procedure for TRO Analysis

1. Method Overview

When pH value is in the range of 6.2-6.5 and having excess potassium iodide, chlorine, hypochlorous, pypocholoride and chloramine would react with DPD to produce red compound. The absorption measurements of this color could be measured by spectrophotometry at wavelength of 515 nm to measure the total residual oxidants.

2. Method References

Determination of free chlorine and total chlorine - Spectrophotometric method using N, N – diethyl - 1, 4 – phenylenediamine (HJ 586-2010).

3. Instruments and Reagents

- Concentrated sulfuric acid: ρ = 1.84 g/mL.
- Potassium iodide (KI): crystal.
- NaClO solution: ρ (Cl_2) ≈ 0.1 g/L, dilute the concentrated NaClO solution to obtain.
- Sulfuric acid solution: c (H_2SO_4) = 1.0 mol/L Carefully add 54.0 mL of the concentrated sulfuric acid into 800 mL water and keep stirring simultaneously. When the solution cools down, move it into 1000 mL volumetric flask and fix the volume by adding water. Mix thoroughly.
- NaOH solution: c (NaOH) = 2.0 mol/L
- NaOH solution: c (NaOH) = 1.0 mol/L
- Stock solution of potassium iodate: ρ (KIO_3) = 1.006 g/L
- Standard solution of potassium iodate I: ρ (KIO_3) = 10.06 mg/L
- Standard solution of potassium iodate II: ρ (KIO_3) = 1.006 mg/L
- Phosphate buffered solution: pH = 6.5
- N, N-diethyl-1, 4-benzene 2 amine sulfate solution (DPD): ρ [NH_2-C_6H_4–$N(C_2H_5)_2$·H_2SO_4] = 1.1 g/L
- Sodium arsenite solution or thioacetamide solution: ρ ($NaAsO_2$) = 2.0 g/L, ρ(CH_3CSNH_2) = 2.5 g/L.
- Visible infrared spectrometer: having 10 mm and 50 mm cuvettes.

4. Analytical Procedures

Free chlorine and total chlorine are not stable, so samples should be test in situ. Otherwise, the samples should be added fixing agent for preservation. It is better to add NaOH solution (1% volume of sample) to brown glass bottle beforehand. When the glass bottle is full of sample, plug the bottle tightly and seal the mouth to avoid contacting with air. If the sample is acidic solution, it should add more NaOH solution to ensure the pH value is greater than 12. Use freezer to transport water sample to laboratory. The water sample should be preserved in dark place at 4°C and should be tested within 5 days.

Calibration curve for high concentration samples: Pipette 0, 1.00, 2.00, 3.00, 5.00, 10.0 and 15.0 mL of standard solution of potassium iodate I (KIO_3) into 100 mL volumetric flask, respectively. Add 50 mL water and 1.0 mL sulfuric acid solution (H_2SO_4) to every volumetric flask. After 1 min, add 1 mL NaOH solution to every volumetric flask and fix the volume by adding water. The mass concentration of chlorine ρ (Cl_2) in each volumetric flask is 0.00, 0.10, 0.20, 0.30, 0.50, 1.00 and 1.50 mg/L, respectively. Add 15.0 mL buffer solution and 5.0 mL DPD into a 250 mL conical flask. In 1 min, add above series of prepared solutions into the conical flask. Mix thoroughly. Use 515

nm of wavelength and 10 mm cuvette to measure absorbance of each solution. It should finish colorimetric analysis within 60 min. Take the absorbance value of corrected blank sample as vertical coordinates and take the corresponding mass concentrations of chlorine $\rho(Cl_2)$ as abscissa to draw the calibration curve.

Calibration curve for low concentration samples: Pipette 0, 2.00, 4.00, 8.00, 12.00, 16.0 and 20.0 mL of standard solution of potassium iodate II (KIO_3) into 100 mL volumetric flask, respectively. Add 50 mL water and 1.0 mL sulfuric acid solution (H_2SO_4) to every volumetric flask. After 1 min, add 1 mL NaOH solution to every volumetric flask and fix the volume by adding water. The mass concentration of chlorine ρ (Cl_2) in each volumetric flask is 0.00, 0.02, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/L, respectively. Add 15.0 mL buffer solution and 1.0 mL DPD into a 250 mL conical flask. In 1 min, add above series of prepared solutions into the conical flask. Mix thoroughly. Use 515 nm of wavelength and 10 mm cuvette to measure absorbance of every solution. It should finish colorimetric analysis within 60 min. Take the absorbance value of corrected blank sample as vertical coordinates and take the corresponding mass concentrations of chlorine $\rho(Cl_2)$ as abscissa to draw the calibration curve.

Sample analysis: add 15.0 mL phosphate buffered solution, 5.0 mL DPD solution, 100 mL water (or the diluted water sample) and 1.0 g KI into 250 mL conical flask. Mix thoroughly. Under the same condition with that of drawing calibration curve, measure the absorbance. Use the absorbance value of corrected blank sample to compute the corresponding mass concentration of chlorine (ρ_2).
Note: when measuring the low concentration sample, it should add 1.0 mL DPD solution.

Use experimental water to replace practical water samples. Following the same procedure, measure the blank samples at the same time.

The mass concentration of total chlorine is computed by the following formula:

$$\rho(Cl_2) = (\rho_2 - \rho_3) \times f$$

where: $\rho(Cl_2)$ = the mass concentration of total chlorine in water sample, mg/L;
ρ_2 = the mass concentration of total chlorine in test sample mg/L;
ρ_3 = the equivalent mass concentration of chlorine when measuring the interference of manganese oxide and Cr VI, mg/L; if there is not manganese oxide and Cr VI in sample, then ρ_3 = 0 mg/L;
f = the dilution factor of test sample.

5. References

Water quality - Determination of free chlorine and total chlorine - Spectrophotometric method using N,N-diethyl -1,4-phenylenediamine (HJ 586, 2010).

Total Residual Oxidant (TRO) analysis LOG sheet

Date: Operator: Approved by:

Sample No.	Time		Absorbance		TRO (mg/L)
	Sampling time	Detection time	Sample absorbance A1	Corrected absorbance Ar	

The volume of water samples (mL):	Low concentration calibration curve:

$\rho(Cl_2) = (\rho_2 - \rho_3) \times f$

$\rho(Cl_2)$—— the mass concentration of total chlorine in water sample, mg/L;

ρ_2 —— the mass concentration of total chlorine in test sample mg/L;

ρ_3 —— the equivalent mass concentration of chlorine when measuring the interference of manganese oxide and Cr VI, mg/L; if there is not manganese oxide and Cr VI in sample, then ρ_3=0 mg/L;

f—— the dilution factor of test sample.

Remarks:

Appendix M Standard Operating Procedure for DO Analysis

Iodometric method is adopted to measure dissolved oxygen in accordance with Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/31).

1. Scope and Application

This method is applicable to dissolved oxygen determination of seawater in ocean and offshore area and river water in river and river mouth.

2. Method Overview

Dissolved oxygen in water sample reacts with manganese chloride and sodium hydroxide to generate brown precipitate of higher valency manganese. In the presence of iodide ions in an acidic solution, liberation of iodine is released which is equivalent to original DO content. Liberation of iodine is then titrated with a standard solution of sodium thiosulfate. DO content can be obtained by conversion.

3. Reagent

3.1 Manganese chloride solution: weigh 210 g manganese chloride ($MnCl_2 \cdot 4H_2O$). Dissolve it into water and dilute to 500 mL.

3.2 Alkaline potassium iodide solution: weigh 250 g sodium hydroxide (NaOH). Dissolve it into 250ml water along with stirring. After it cools down, add 75 g potassium iodide (refer to 3.6) and dilute to 500 mL. Store it in brown reagent bottle with rubber plug.

3.3 Sulfuric acid solution (1+1): Carefully add the same volume of concentrated sulfuric acid (H_2SO_4: 1.84 g/mL) to the same volume of water while stirring and make it mixed. Store it in reagent bottle.

3.4 Sodium thiosulfate solution [c ($Na_2S_2O_3 \cdot 5H_2O$) = 0.01 mol/L].

3.5 Starch solution (5 g/L).

3.6 Potassium iodide (KI): chemical pure.

3.7 Standard potassium iodate solution [c ($1/6KIO_3$) = 0.0100 moI/L]: weigh 3.567 g potassium iodate (KIO_3, GR, pre-bake for 2 hours at 120°C and cool in the silica gel drier). Dissolve it into water and shift it to 1000 mL volumetric flask totally. Fix the volume by adding water and mix the solution. Store it in cold and dark place. Period of validity is one month. Measure 10.00 mL and dilute to 100 mL with water when it is used.

4. Instruments and Equipment

Water sample bottle: 125 mL, brown ground glass bottles with tapered cork. Grinding mouth should be tight and the capacity needs to be correction.

Glass tube: Diameter of 5-6 mm, length of 12 cm

Latex tube: Same diameter with the glass tube, length of 20-30 cm

Dissolved oxygen burette: 25 mL, Scale graduation of 0.05 mL

Magnetic stirrer: Rotational speed can be adjusted to (150-400) r/min

Glass magnetic rotor: Diameter of about 3~5mm. Length of 25 mm

Conical flask: 250 mL

Iodine bottle: 250 mL

Graduated cylinder: 100 mL

Beaker: 500 mL, 1000 mL

Double inflating ball

Brown reagent bottle: 500 mL, 2500 mL

Bottle- top dispenser: 5 mL

Transfer pipet: 20 mL

Common instruments and equipment in general laboratory.

5. Procedure

5.1 Water sample fixing: Open the cork of water sample, and immediately add 1.0 mL manganese chloride solution and 1.0 mL alkaline potassium iodide solution orderly to water sample using bottle- top dispenser (tube tip is inserted under liquid surface). Tie the cork tightly (bubble is forbidden in the bottle) and hold it. Turn the bottle upside down for no less than 20 times.

5.2 Determination steps

a) wait for about 1 hour or precipitation is produced completely, titration can be started;
b) Pour the supernatant of sample bottle into the 250 mL conical flask, and add 1.0 mL sulfuric acid solution immediately. Tie the cork tightly and shake the bottle until precipitates are dissolved totally;
c) Pure the whole solution in the sample bottle into the conical flask. Place it on the magnetic stirrer, and stir immediately. Titrate with the calibrated sodium thiosulfate solution;
d) When the test solution looks yellow, add 1 ml starch solution. Continue to titrate until blue has just faded. Swing original water sample bottle with few test solution in conical flask, and then pour it back to the conical flask. Continue to titrate until colorless. Wait for 20 seconds. If the test solution doesn't look light blue, titration endpoint is reached. Record the volume of sodium thiosulfate solution for titration in datasheet.

5.3 Blank test

Measure 100 mL seawater and mix the water with 1.0 mL sulfuric acid solution, 1.0 mL alkaline potassium iodide solution and 1.0 mL manganese chloride solution. Place the solution aside for 10 minutes. Add 1 mL starch solution. Mix thoroughly. Meanwhile, if the solution looks light blue, continue to titrate with sodium thiosulfate solution. If the used amount of sodium thiosulfate

solution is more than 0.1 mL, check the reliability of potassium iodide and manganese chloride reagent and prepare the reagent again. If the used amount of sodium thiosulfate solution is no more than 0.1 mL or the solution doesn't look light blue after adding starch solution, and the solution looks blue immediately once 1 drop of standard potassium iodate solution is added, reagent blank can be ignored. Blank test should be done one time for each batch of new prepared reagent.

6. Computing and Reporting

6.1 Concentration of dissolved oxygen in water sample can be calculated as follows:

$$\rho_{O_2} = \frac{c \times V \times 8}{V_0} \times 1000$$

Where:
ρ_{02} = concentration dissolved oxygen in water sample, mg/L;
c = concentration of sodium thiosulfate solution, mg/L;
V = volume of sodium thiosulfate solution used for titration of the sample, mL;
V_0 = actual volume of the water sample used for titration (=volume of water sample bottle – fixative volume used to fix the water sample), mL;

6.2 Dissolved oxygen saturation can be calculated as follows:

$$\text{Dissolved oxygen saturation} = \rho_{02} / \rho_{02}' \times 100$$

Where:
ρ_{02}=measured oxygen content, the unit is milligrams per liter (mg/L);
ρ_{02}'=oxygen saturation concentration in seawater at water temperature and salinity on the scene, the unit is milligrams per liter (mg/L).

7. Attentions

- Unless otherwise stated, all reagents used in this method are of analytical reagent (AR) grade and all water used is distilled water or equivalent pure water;
- Dissolved water sample bottle needs capacity calibration: Fill the water sample bottle with full of distilled water. Tie the cork, dry the bottle and then weigh it. This weight minus the weight of dry empty bottle and is divided by the density of distilled water, and then the capacity of the bottle can be achieved. Record the number of the bottle and the corresponding measurement results of capacity for future reference;
- When endpoint is near, the titration speed should not be too slow; otherwise color changes would be insensitive at the endpoint. If the solution looks amaranth before reaching the endpoint, it shows the starch solution has been deteriorated and needs to be prepared again;
- If water sample contains oxidizing substances, it can separate iodine so as to produce the positive interference. If it contains reducing substances, it can consume iodine so as to produce the negative interference.

DO analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH		
Instrument	Burette, Magnetic stirrer	Detection date and time	

Test Results

Sample ID	Sample volume (V_0, mL)	$Na_2S_2O_3$ solution (V, mL)			DO level (mg/L)
		1	2	Mean	

Calculation $\rho_{o_2} = \frac{c \times V \times 8}{v_0} \times 1000$ $c(Na_2S_2O_3)$=

Remarks:

Appendix N Standard Operating Procedure for Salinity Analysis

The testing method use SYA2-2 salimeter to determine salinity of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/29.1).

1. Scope and Application

This method is applicable to salinity determination of seawater on land or in the laboratory on board.

2. Method Overview

Use inductive salinity meter for determination.

3. Reagent

Standard seawater.

4. Instruments and Equipment

Main technical indicators of inductive salinity meter: conductivity ratio 0.07-1.2; measurement accuracy is 0.01; measurement precision is 0.003; salinity resolution is 0.001; accuracy of temperature measuring of electrical bridge is 0.5°C.

5. Procedure

5.1 Preparation: Place the water sample until the temperature difference between the sample and standard seawater is within ±2°C to prepare for measurement.

5.2 Temperature and salinity measurement inspection

5.2.1 Turn the temperature-salinity switch to “temperature measurement”. Compare the temperature reading with the room temperature. If the deviation is lower than ±1°C, the temperature bridge operates normally.

5.2.2 Tighten the drainage knob below the water cup. Place the seawater whose salinity is known to the inlet below the conductivity cell and turn the clock of conductivity cell to “inlet”. Open the air pump switch. Press the gas hole on the water cap tightly with the middle finger of the left hand, and at that time, the seawater will flow into the conductivity cell slowly. When a little seawater overflow from the outlet of conductivity cell to the water cup, turn off the cock of conductivity cell immediately, release the finger and close the air pump. The conductivity cell is filled with the seawater at that time.

5.3 Calibration

5.3.1 Fill the conductivity cell with the standard seawater slowly. Wash the cell for 1-2 times, and then measure the temperature of standard seawater and write down the data on datasheet.

5.3.2 Look up the corresponding R2 value according to the temperature conversion table on the instrument panel. Rotate R2 knob to this value and record R2 value.

5.3.3 Shift the temperature-salinity switch to “salinity measurement” and adjust R1 knob to make zero-header point at “zero”. Stop stirring and drain water. Repeat filling adjustment until the reading repeats, then the calibration is completed. Record the R1 value.

5.4 Water sample measurement

Start up the air pump. Pipette the water sample into the conductivity cell and wash for 1-2 times. When the water sample overflows from the conductivity cell, turn off the inlet cock of conductivity cell immediately, cut down the power of the air pump and start mixing. Turn temperature-salinity switch to “temperature measurement”. Measure the temperature of the seawater sample and write down the data in the datasheet. Shift temperature-salinity switch to “salinity measurement” and adjust Rt knob to make zero-header point at “zero”. Stop stirring and drain the water sample in the conductivity cell. If the change of last digits of conductivity ratio knob in two measurements is less than 6, these two measurements are regarded as repeat. Record the conductivity ratio R1 value of the seawater sample.

6. Computing and Reporting

According to the formula, programming should be relied on for calculation. The result should display three digits after the decimal point.

7. Attentions

- 250 mL sampling bottle and the plug should be washed for 3 times with the same water sample before use. The used sampling bottle should be filled with a little seawater but drained the water at next sampling time;
- When fill the conductivity cell with the seawater sample, avoiding the bubbles in the conductivity cell. If there are bubbles, the reading will be lower in general. At that time, the sample should be re-filled and measured again;
- When fill the conductivity cell with the seawater sample, drain the residual water sample inside inflow tube and dry the tube. After that, measure the sample according to the steps described. Otherwise, the residual water will pollute the water sample.

Salinity analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH	Detection date and time	
Instrument	Salimeter		
Instrument model	SYA2-2		
Standard value of standard seawater:	R_{15} value of standard seawater:	Control standard seawater (detection):	

Test Results

Sample ID	Salinity (PSU)	Sample ID	Salinity (PSU)

Remarks:

Appendix O Standard Operating Procedure for Temperature Analysis

The testing method use Surface Water Thermometer to determine temperature of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/25.2).

1. Analysis method

When using the surface water thermometer, tie the haul loop on top of the metal pipe with rope firstly. Use bucket to collect water sample. Put the surface water thermometer into the bucket during measurement. After 1-2 minutes, empty the water in the bucket and thermometer tube. Collect the water sample and put the thermometer into the bucket again. Read the temperature after responding for 3 minutes and read the temperature again after another 1 minute. When the air temperature is higher than the water temperature, calibrate the smaller one of the readings to get the measure value of surface water temperature. On the contrary, calibrate the bigger one of the readings to get the measure value of surface water temperature.

2. Attentions

- When reading the data, the sight line and the top of the capillary of the surface water thermometer should be at the same level. Direct sunlight should be avoided.
- The bucket is better to be made of heat-insulation materials. The capacity is about 5-10 L.
- Surface water thermometer must have periodic verifications according to the specification.

Temperature analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH	Detection date and time	
Instrument	Thermometer		
Instrument model			

Test Results

Sample ID	Temperature (°C)	Sample ID	Temperature (°C)

Remarks	Corrected value of thermometer			
	Indicating value (°C)	0	20	40
	Corrected value (°C)	0.36	0.46	0.62